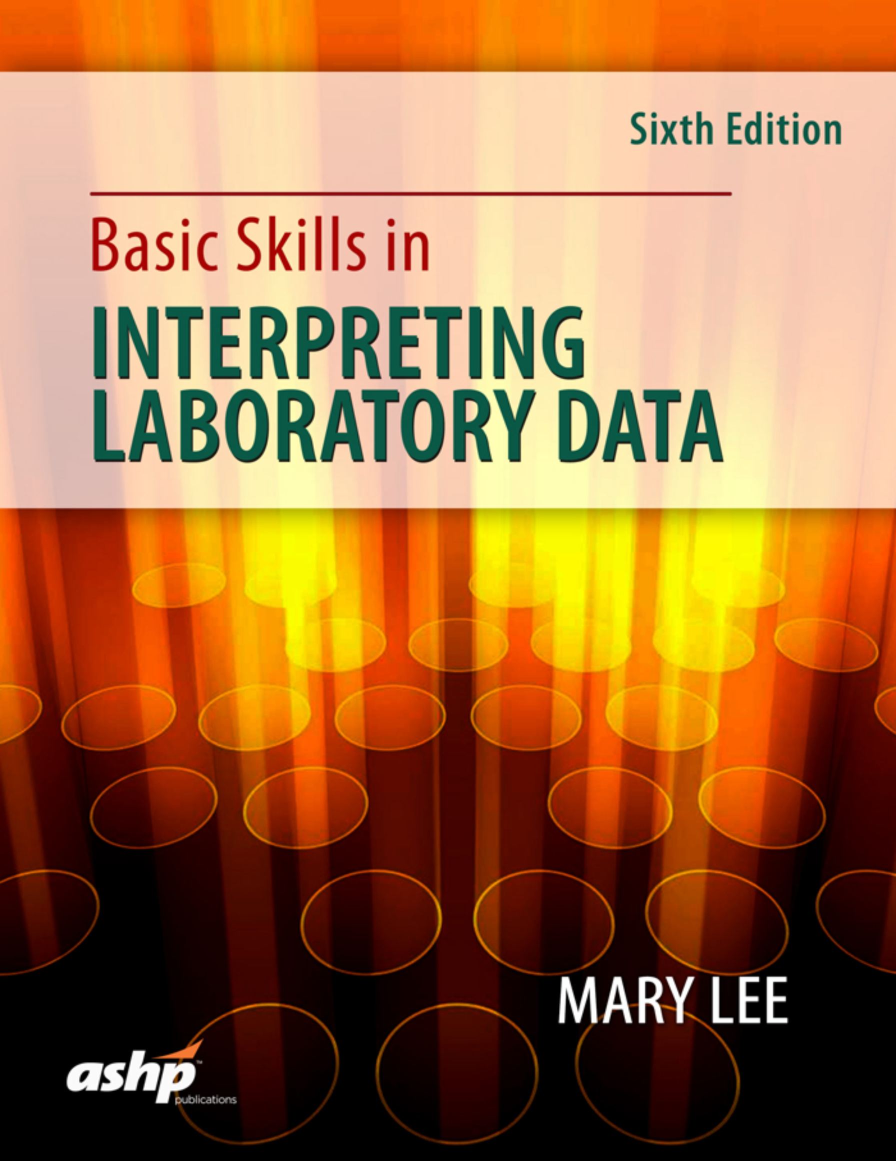

Sixth Edition

Basic Skills in
INTERPRETING LABORATORY DATA

MARY LEE

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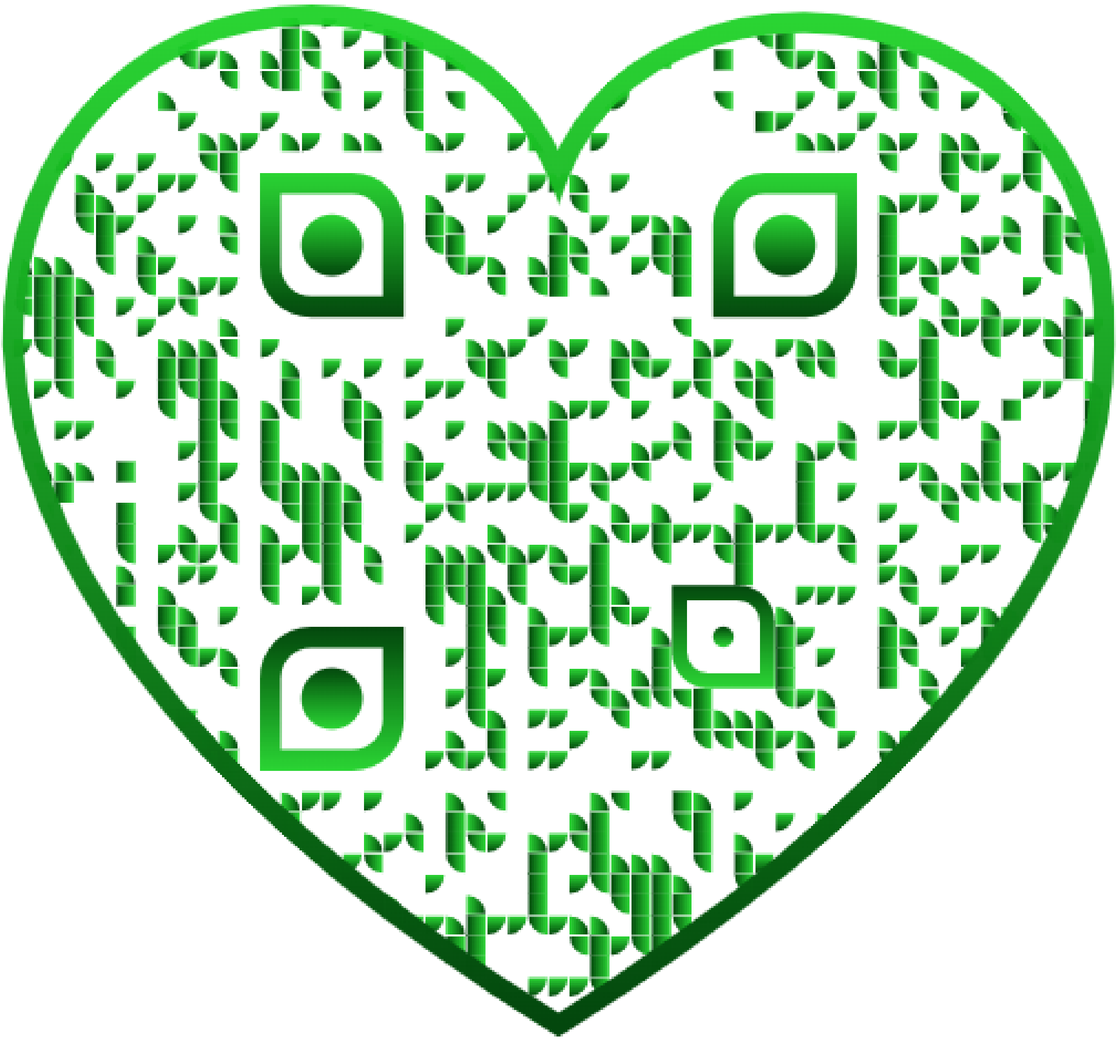

Sixth Edition

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INTERPRETING LABORATORY DATA

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DEDICATION

This book is dedicated to all of the chapter authors and reviewers, whose commitment to the education of future health professional students is evident in all that they do.

Mary Lee

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Mary Lee

PREFACE

The last four editions of *Basic Skills in Interpreting Laboratory Data* have been made possible by the dedicated chapter authors, reviewers, and the publishing staff at the American Society of Health-System Pharmacists. It has been my honor to serve as the editor and to work with this team.

For this sixth edition, approximately 90% of the lead authors have served in this capacity for the earlier editions with some exceptions. Paul O. Gubbins, PharmD, and Heather Lyons-Burney, PharmD, joined as the lead authors of a new chapter on Point-of-Care Testing, and Nicholas M. Moore, MS, MLS (ASCP), updated the chapter on Introduction to Common Laboratory Assays and Technology. All of the lead authors are established clinicians and/or experienced faculty at colleges of pharmacy or medicine, which enhance the quality of the chapter content.

A whole new group of reviewers has joined this project, and many reviewers are board-certified or established experts. Their specialty knowledge and scrutiny of the chapter content have helped to ensure that each chapter is up-to-date and content is relevant to clinical practice. As you use this book, you will find that the sixth edition includes updated chapter content with references, and almost all of the chapters have at least one new Minicase and Learning Point. In addition, the Abbreviations in the front of the book and the Glossary in the back have been expanded for reader convenience.

Significant and notable new chapter content:

1. Hematology: Blood Coagulation Tests includes expanded sections on laboratory tests to monitor direct thrombin inhibitors, direct oral anticoagulants, and low molecular weight heparin.
2. Hematology: Red and White Blood Cell Tests includes a discussion of cell types, associated cluster of differentiation epitopes or targets, and FDA-approved targeted therapies.
3. Infectious Diseases includes an expanded section on molecular diagnosis of specific viral nucleic acids and 1,3-β-glucan detection of fungi.
4. Liver and Gastroenterology Tests includes a new section on laboratory tests to diagnose and monitor hemochromatosis.
5. Interpretation of Serum Drug Concentrations includes information on new medications that have become commercially available since the last edition.
6. Men's Health includes an expanded section on PSA testing for screening, staging, and monitoring treatment of prostate cancer.

Suggestions for using this book efficiently:

- For a general overview of the laboratory tests for various organ systems or types of diseases, use the table of contents to identify the most appropriate section or chapter(s). The chapters are grouped into three major sections: Basic Concepts and Test Interpretations, System Disorders and Diagnostic Tests, and Tests for Special Populations. By reading the section or a chapter from start to finish, you get a detailed summary of the laboratory tests used to evaluate that organ system or disease, why the test is used, what a normal value range is for the test, and how to interpret an abnormal laboratory test result. Minicases guide the reader through common clinical scenarios about ordering appropriate laboratory tests, interpreting results, managing patients, and addressing spurious laboratory tests. Learning points conclude each chapter and highlight key concepts about the laboratory tests. Using the book in this way will be helpful, especially when used as a companion to a disease state management course, a pharmacotherapeutics course, or a course that prepares students for full-time clinical rotations.
- For information on a specific laboratory test, use the alphabetical index to locate the test, and then go to the page(s) to access the following information: the purpose of the test; how the test result relates to the pathophysiology of a disease or the physiologic function of a cell or organ;

the normal range for the test; causes for an abnormal test result; and causes of false-positive or false-negative results. This approach will be most useful in the clinical management of a patient.

- Quickview charts are provided for some of the most common laboratory tests. These charts are standardized template presentations of information that allow readers to quickly learn about a specific laboratory test (e.g., what the test is used for, what a normal result is, and causes of an abnormal result). This approach also will be most useful in the clinical management of a patient, but the Quickview content should be supplemented with the in-depth information in the chapters about a particular laboratory test. Although this book does not provide Quickview charts for all of the laboratory tests discussed, readers can refer to other clinical laboratory test handbooks, such as ASHP's *Interpreting Laboratory Data: A Point-of-Care Guide*.

The authors, reviewers, and I hope that *Basic Skills in Interpreting Laboratory Data* is useful to your practice.

Mary Lee
May 2017

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ABBREVIATIONS

μm	micrometer
1,25-DHCC	1,25-dihydroxycholecalciferol
17-OHP	17α-hydroxyprogesterone
^{201}TI	thallium-201
2,3 DPG	2,3-diphosphoglycerate
25-HCC	25-hydroxycholecalciferol
3SR	self-sustained sequence replication
5HT	serotonin
6-AM	6-acetylmorphine
6MWT	6-minute walk test
^{99m}Tc	technetium-99m
^{201}Tl	thallium-201 (radio isotope)
α_1AC	α1-antichymotrypsin
A-G6PD	glucose-6 phosphate dehydrogenase variant
A1c	glycosylated hemoglobin
A2M, α2M	α2-macroglobulin
AACE	American Association of Clinical Endocrinologists
AAG	α1-acid glycoprotein
ABG	arterial blood gas
ACA	anticentromere antibody
ACC	American College of Cardiology
ACCF	American College of Cardiology Foundation
ACCP	American College of Clinical Pharmacy
ACCP	anticyclic citrullinated peptide
ACE	angiotensin-converting enzyme
ACE-I	angiotensin-converting enzyme inhibitor
ACPA	anticitrullinated protein antibody
ACR	albumin-to-creatinine ratio; American College of Rheumatology
ACS	acute coronary syndrome
ACT	activated clotting time; α1-coded testing
ACTH	adrenocorticotropic hormone (corticotropin)
ADA	American Diabetes Association
ADAM	androgen deficiency in aging males
ADCC	antibody-dependent cellular cytotoxicity
ADH	antidiuretic hormone
ADME	absorption, distribution, metabolism, excretion
ADP	adenosine diphosphate
AFB	acid-fast bacilli
AFP	α-fetoprotein
AG	anion gap
AGPA	allergic granulomatosis with polyangiitis
AHA	American Heart Association
AIDS	acquired immunodeficiency syndrome
ALK	anaplastic lymphoma kinase
ALL	acute lymphoblastic leukemia
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AMA	antimitochondrial antibody
AMI	acute myocardial infarction
AML	acute myelogenous leukemia
ANA	antinuclear antibody
ANCA	antineutrophil cytoplasmic antibody
ANF	atrial natriuretic factor
ANP	atrial natriuretic peptide
anti-HAV IgG	IgG antibody against hepatitis A virus
anti-HAV IgM	IgM antibody against hepatitis A virus
anti-HBc	antibody to hepatitis B core antigen
anti-HbeAg	antibody to hepatitis B extracellular antigen
anti-HBs	antibody to hepatitis B surface antigen
anti-HCV	antibody against HCV antigen
anti-HD	antibody against hepatitis D
APC	activated protein C
APC	antigen-presenting cell
apoB	apolipoprotein B
APS	antiphospholipid antibody syndrome
aPTT	activated partial thromboplastin time
ARB	angiotensin receptor blocker
ASA	aspirin
ASCO	American Society of Clinical Oncology
ASCVD	atherosclerotic cardiovascular disease
AST	aspartate aminotransferase
AT	antithrombin
ATP	adenosine triphosphate
ATP-K	adenosine triphosphate potassium
ATP	Adult Treatment Panel
ATP III	Adult Treatment Panel III
ATS	American Thoracic Society
AUA	American Urological Association
AUA-SI	American Urological Association Symptom Index
AUC	area under the (serum concentration time) curve
AV	atrioventricular
AVP	arginine vasopressin
B&B	Brown and Brenn
B2M	β2-microglobulin
BAL	bronchial alveolar lavage; bronchoalveolar lavage
BAMT	blood assay for *Mycobacterium tuberculosis*
BBT	basal body temperature
BCG	Bacillus Calmette-Guérin
bDNA	branched-chain DNA

BGMK-hDAF	buffalo green monkey kidney cell line decay accelerating factor
BHI	brain heart infusion
BHR	bronchial hyper-responsiveness
BID	twice daily
BMI	body mass index
BMP	basic metabolic panel
BNP	brain natriuretic peptide
BP	blood pressure
BPH	benign prostatic hyperplasia
BPSA	benign form of prostate-specific antigen
BPT	bronchial provocation testing
BRAF	v-Raf murine sarcoma viral oncogene homolog B1
BSA	body surface area
BSL	biosafety level
BT	bleeding time
BUN	blood urea nitrogen
C. difficile	*Clostridium difficile*
C3	complement protein 3
C4	complement protein 4
CA	cancer antigen
CA	carbonic anhydrase
CABG	coronary artery bypass graft
CA_{corr}	corrected serum calcium level
CAD	coronary artery disease
CAH	congenital adrenal hyperplasia
CAN2	chromID Candida agar
cANCA	cytoplasmic antineutrophil cytoplasmic antibody
CAP	College of Pathologists
CAP	community-acquired pneumonia
CAT	computerized axial tomography
CA_{uncorr}	uncorrected serum calcium level (or actual measured total serum calcium)
CBC	complete blood count
CCFA	cycloserine cefoxitin fructose agar
CCNA	cell cytotoxicity neutralization assay
CCP	cyclic citrullinated peptide
CCR5	chemokine coreceptor 5
cCRP	cardiac C-reactive protein
CCT	cardiac computed tomography
cd	candela
CD	clusters of differentiation
CDC	Centers for Disease Control and Prevention
CDR	complementarity-determining regions
CE	capillary electrophoresis
CEA	carcinoembryonic antigen
CEDIA	cloned enzyme donor immunoassay
CETP	cholesteryl ester transfer protein
CF	complement fixation
CFTR	cystic fibrosis transmembrane conductance regulator
CFU, cfu	colony-forming units
CFW	calcofluor white
CGE	capillary gel electrophoresis
CH_{50}	complement hemolytic 50%
CHD	coronary heart disease
CHF	congestive heart failure
CI	chemical ionization
CIS	combined intracavernous injection and stimulation
CK	creatine kinase
CK-BB	creatine kinase isoenzyme BB
CK-MB	creatine kinase isoenzyme MB
CK-MM	creatine kinase isoenzyme MM
CK1	creatine kinase isoenzyme 1
CK2	creatine kinase isoenzyme 2
CK3	creatine kinase isoenzyme 3
CKD	chronic kidney disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CLIA-88	Clinical Laboratory Improvement Amendments of 1988
CLIA	Clinical Laboratory Improvement Amendments
CLL	chronic lymphocytic leukemia
CLSI	Clinical and Laboratory Standards Institute
cm	centimeter
CMA	cornmeal agar
C_{min}	minimum concentration (of a drug)
CML	chronic myelogenous leukemia
CMP	comprehensive metabolic panel
CMR	cardiac magnetic resonance
CMV	cytomegalovirus
CNA	colistin-nalidixic acid
$C_{normalized}$	normalized total concentration
CNP	c-type natriuretic peptide
CNS	central nervous system
CO	carbon monoxide; cardiac output; cyclooxygenase
CO_2	carbon dioxide
CO-Hgb	carboxyhemoglobin
COP	colloid osmotic pressure
COPD	chronic obstructive pulmonary disease
CPE	cytopathic effect
CPK	creatine phosphokinase
CPPD	calcium pyrophosphate dihydrate
cPSA	complexed PSA
CrCl	creatinine clearance
CREST	syndrome characterized by **c**alcinosis, **R**aynaud disease, **e**sophageal motility disorder, **s**clerodactyly, and **t**elangiectasias
CRH	corticotrophin-releasing hormone
CRP	C-reactive protein
CSF	cerebrospinal fluid
$C_{ss, avg}$	average steady-state concentration (of a drug)
CT	computed tomography
cTnC	cardiac-specific troponin C
cTnI	cardiac-specific troponin I

cTnT	cardiac-specific troponin T
CVD	cardiovascular disease
CX	circumflex
CXCR4	CXC chemokine coreceptor
CYP	cytochrome P450 drug metabolizing enzymes
CYP2C19	cytochrome P450 2C19 enzyme
CYP2D6	cytochrome P450 2D6 enzyme
CYP3A4	cytochrome P450 3A4 enzyme
CYP450	cytochrome P450 enzyme
CYP4F2	cytochrome P450 4F2 enzyme
CZE	capillary zone electrophoresis
D&C	dilation and curettage
D5W	5% dextrose in water
DASH	**d**ietary **a**pproaches to **s**top **h**ypertension
DAT	direct agglutination test
DAT	direct antibody test
DCCT	Diabetes Control and Complications Trial
DCP	des-gamma-carboxyprothrombin
DDAVP	desmopressin
dTT	dilute thrombin time
DDT	dichlorodiphenyltrichloroethane
DFA	direct fluorescent antibody
DHA	docosahexaenoic acid
DHEA	dehydroepiandrostenedione or dehydroepiandrosterone
DHEAS	dehydroepiandrosterone sulfate
DI	diabetes insipidus
DIC	disseminated intravascular coagulation
DIM	dermatophyte identification medium
DKA	diabetic ketoacidosis
dL	deciliter
DLCO	diffusing capacity of the lung for carbon monoxide
DM	diabetes mellitus
DNA	deoxyribonucleic acid
DNP	dendroaspis natriuretic peptide
DO_2	oxygen delivery
DOAC	direct oral anticoagulant
DPD	dihydropyrimidine dehydrogenase
DPP-4	dipeptidyl peptidase-4
dsDNA	double-stranded DNA
DST	dexamethasone suppression test
DTI	direct thrombin inhibitor
DTM	dermatophyte test medium
E2	estradiol
EBM	esculin base medium
EBV	Epstein-Barr virus
ECD	energy coupled dye
ECG	electrocardiogram
ECMO	extracorporeal membrane oxygenation
ECT	ecarin clotting time
ECW	extracellular water
ED	emergency department
EDTA	ethylenediaminetetraacetic acid
EGFR	epidermal growth factor receptor
eGFR	estimated glomerular filtration rate
EF	ejection fraction
EI	electron ionization
EIA	enzyme immunoassay
EIB	exercise- or exertion-induced bronchospasm
EKG	electrocardiogram
ELISA	enzyme-linked immunosorbent assay
ELVIS	enzyme-linked virus-inducible system
EM	electron microscopy
EMB	eosin methylene blue
EMIT	enzyme-multiplied immunoassay technique
EOF	electroosmotic force
EPA	eicosapentaenoic acid
EPS	expressed prostatic secretions
ER	estrogen receptor
ERS	European Respiratory Society
ERV	expiratory reserve volume
ESA	erythrocyte-stimulating agent
ESBL	extended-spectrum β-lactamase
ESC	European Society of Cardiology
ESI	electrospray ionization
ESR	erythrocyte sedimentation rate
ESRD	end-stage renal disease
Etest	epsilometer test
ETIB	enzyme-linked immunoelectrotransfer blot
EU	ELISA units
EUCAST	European Committee on Antimicrobial Susceptibility Testing
EULAR	European League Against Rheumatism
FA	fluorescent antibody
Fab	fraction antigen-binding
FAB	fast atom bombardment
FAB	French-American-British
FACS	fluorescence-activated cell sorting
FALS	forward-angle light scattering
FANA	fluorescent antinuclear antibody
FDA	Food and Drug Administration
FDP	fibrin degradation product
$FEF_{25–75}$	forced expiratory flow at 25% to 75% of vital capacity
FEF	forced expiratory flow
FE_{Na}	fractional excretion of sodium
FENO	fractional exhaled nitric oxide
FEV_1	forced expiratory volume in 1 second
FiO_2	fraction of inspired oxygen
FISH	fluorescence in situ hybridization
FITC	fluorescein isothiocyanate
fL	femtoliter
FM	Fontana-Masson
FN	false negative
FP	false positive
FPG	fasting plasma glucose
FPIA	fluorescence polarization immunoassay

fPSA	free prostate specific antigen
FRC	functional residual capacity
FSH	follicle-stimulating hormone
FTA-ABS	fluorescent treponemal antibody absorption
FVC	forced vital capacity
FWR	framework regions
g	gram
G-CSF	granulocyte colony–stimulating factor
G6PD	glucose-6 phosphate dehydrogenase
GA	gestational age
GADA	glutamic acid decarboxylase autoantibodies
GAP	group A streptococcus
GAS	group A streptococci
GC	gas chromatography
GC-MS	gas chromatography and mass spectrometry
GERD	gastroesophageal reflux disease
GF	Gridley fungus
GFR	glomerular filtration rate
GGT, GGTP	gamma-glutamyl transferase; gamma-glutamyl transpeptidase
GHB	gamma-hydroxybutyrate
GI	gastrointestinal
GIP	glucose-dependent insulinotropic peptide
GLC	gas liquid chromatography
GLP-1	incretin hormones glucagon-like peptide-1
GLUT	glucose transporter
GM-CSF	granulocyte/macrophage colony-stimulating factor
GMS	Gomori methenamine silver
GnRH	gonadotropin-releasing hormone
GOLD	Global Initiative for Chronic Obstructive Lung Disease
gp	glycoprotein
GPA	granulomatosis with polyangiitis
GTF	glucose tolerance factor
H&E	hematoxylin and eosin
H. Pylori	*Helicobacter pylori*
HAAg	hepatitis A antigen
HAP	hospital-acquired pneumonia
HAV	hepatitis A virus
Hb; hgb	hemoglobin
HbA1c	glycated hemoglobin
HBcAg	hepatitis B core antigen
HBeAg	hepatitis B extracellular antigen
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
hCG	human chorionic gonadotropin
HCO_3^-	bicarbonate
HCT, Hct	hematocrit
HCV	hepatitis C virus
HDAg	hepatitis D antigen
HDL	high-density lipoprotein
HDL-C	high-density lipoprotein cholesterol
HDV	hepatitis D virus
HER-1	human epidermal growth factor receptor 1
HER-2	human epidermal growth factor receptor 2
HEV	hepatitis E virus
HF*p*EF	heart failure with preserved ejection fraction
HF*r*EF	heart failure with reduced ejection fraction
HGA	human granulocytic anaplasmosis
Hgb	hemoglobin
HHS	hyperosmolar hyperglycemic state
HIPA	heparin-induced platelet activation
HIT	heparin-induced thrombocytopenia
HIV	human immunodeficiency virus
HIV-1	human immunodeficiency virus type 1
HLA	human leukocyte antigen
HLA-B27	human leukocyte antigen B27
HLA-DQ	human leukocyte antigen coded DQ genes
HLAR	high-level aminoglycoside resistance
HME	human monocytic ehrlichiosis
HMG-CoA	3-hydroxy-3-methyl-glutaryl-coenzyme A
HMWK	high-molecular weight kininogen
HPA	hypothalamic pituitary axis
HPF	high-power field
HPLC	high-performance (or pressure) liquid chromatography
HPV	human papillomavirus
HR	heart rate
hr	hour
hs-CRP	high-sensitivity C-reactive protein
HSG	hysterosalpingogram, hysterosalpingography
hsTnI	high-sensitivity troponin I
hsTnT	high-sensitivity troponin T
HSV	herpes simplex virus
Ht	height
HTN	hypertension
I	intermediate
IA	immunoassay
IA-2A	insulinoma-associated-2 autoantibodies
IAA	insulin autoantibodies
IAT	indirect antibody test
IBW	ideal body weight
IC	inspiratory capacity
IC_{50}	inhibitory concentration 50%
IC_{90}	inhibitory concentration 90%
ICA	immunochromatographic assay
ICA	islet cell cytoplasmic autoantibodies
ICTV	International Committee on Taxonomy of Viruses
ICU	intensive care unit
ICW	intracellular water
ID	immunodiffusion
IDC	International Diabetes Center
IDL	intermediate-density lipoproteins
IDMS	isotope dilution mass spectrometry
IFA	immunofluorescence assay; indirect fluorescent antibody

IFN-γ	interferon gamma
IgA	immunoglobulin A
IgD	immunoglobulin D
IgE	immunoglobulin E
IgG	immunoglobulin G
IgM	immunoglobulin M
IHC	immunohistochemistry
IHD	ischemic heart disease
IIEF	International Index of Erectile Function
IIM	idiopathic inflammatory myopathy
IMA	inhibitory mold agar
INR	international normalized ratio
IP	interphalangeal
iPSA	inactive PSA
IPSS	International Prostate Symptom Score
IQ	inhibitory quotient
IRMA	immunoradiometric assay
IRV	inspiratory reserve volume
ISE	ion-selective electrode
ISI	International Sensitivity Index
ITP	idiopathic thrombocytopenic purpura
IV	intravenous
J	joule
JIA	juvenile idiopathic arthritis
JRA	juvenile rheumatoid arthritis
JVP	jugular venous pressure
k	constant of proportionality
K	kelvin
K_{corr}	corrected serum potassium level
KDIGO	Kidney Disease Improving Global Outcomes
kg	kilogram
KIMS	kinetic interaction of microparticles in solution
Km	Michaelis constant
KOH	potassium hydroxide
KRas	V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog
K_{uncorr}	uncorrected serum potassium level (or actual measured serum potassium)
L	liter
LA	latex agglutination
La/SSB	La/Sjögren syndrome B
LAD	left anterior descending
LBBB	left bundle branch block
LC	liquid chromatography
LCAT	lecithin cholesterol acyltransferase
LCR	ligase chain reaction
LDH	lactate dehydrogenase
LDH1	lactate dehydrogenase isoenzyme 1
LDH2	lactate dehydrogenase isoenzyme 2
LDH3	lactate dehydrogenase isoenzyme 3
LDH4	lactate dehydrogenase isoenzyme 4
LDH5	lactate dehydrogenase isoenzyme 5
LDL	low-density lipoprotein

LDL-C	low-density lipoprotein cholesterol
LE	lupus erythematosus
LFT	liver function test
LH	luteinizing hormone
LHRH	luteinizing hormone–releasing hormone
LIS	laboratory information system
LMP	last menstrual period
LMWH	low molecular weight heparin
Lp(a)	lipoprotein(a)
Lp-PLA$_2$	lipoprotein-associated phospholipase A$_2$
LPL	lipoprotein lipase
LSD	lysergic acid diethylamide
LTA	light transmittance aggregometry
LUTS	lower urinary tract symptoms
LVEF	left ventricular ejection fraction
m	meter
m^2	meters squared
MAbs	monoclonal antibodies
Mac	MacConkey
MAC	membrane attack complex
MAC	*Mycobacterium avium* complex
MALDI	matrix-assisted laser desorption/ionization
MALDI-TOF	matrix-assisted laser desorption ionization time-of-flight
MAP	mitogen-activated protein
MAT	microagglutination test
MBC	minimum bactericidal concentration
MBP	mannose-binding protein
mcg	microgram
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MCP	metacarpophalangeal
MCT	medium chain triglycerides
MCTD	mixed connective tissue disease
MCV	mean corpuscular volume
MDMA	3,4-methylenedioxy-N-methamphetamine (Ecstasy)
MDR	multidrug resistant
MDRD	Modification of Diet in Renal Disease
MDx	molecular diagnostics
mEq	milliequivalent
mg	milligram
MHA	Mueller-Hinton agar
MHA-TP	microhemagglutination *Treponema pallidum*
MHC	major histocompatibility complex
MI	myocardial infarction
MIC	minimum inhibitory concentration
MIC_{50}	MIC value representing 50% of a bacterial population
MIC_{90}	MIC value representing 90% of a bacterial population
MIF	microimmunofluorescence
min	minute

mL	milliliter
mm	millimeter
mm^3	cubic millimeter
mmol	millimole
mTOR	mammalian target of rapamycin
moAb	monoclonal antibody
mol	mole
MOTT	mycobacteria other than tuberculosis
MPO	myeloperoxidase
MPV	mean platelet volume
MRI	magnetic resonance imaging
mRNA	messenger ribonucleic acid
MRO	medical review officer
MRP1	multidrug resistant protein 1
MRP2	multidrug resistant protein 2
MRP3	multidrug resistant protein 3
MRSA	methicillin-resistant *Staphylococcus aureus*
MS	mass spectrometry
MSSA	methicillin-susceptible *Staphylococcus aureus*
mTOR	mammalian (or mechanistic) target of rapamycin
MTP	metatarsophalangeal
N	newton
NA	nucleic acid
NAAT	nucleic acid amplification test
NACB	National Academy of Clinical Biochemistry
NAEPP	National Asthma Education Prevention Program
NASBA	nucleic acid sequence-based amplification
NASH	nonalcoholic steatohepatitis
NCCB	nondihydropyridine calcium channel blocker
NCEP	National Cholesterol Education Program
ng	nanogram
NHL	Non-Hodgkin lymphoma
NK cells	natural killer (T) lymphocytes
NKDEP	National Kidney Disease Education Program
NKF KDOQI	National Kidney Foundation Kidney Disease Outcomes Quality Initiative
NLA	National Lipid Association
nm	nanometer
NNRTI	non-nucleoside reverse transcriptase inhibitor
NNS	number needed to screen
NQO1	NADPH quinone dehydrogenase 1
NQMI	non Q-wave myocardial infarction
NRTI	nucleoside reverse transcriptase inhibitor
NSAID	nonsteroidal anti-inflammatory drug
NSCLC	non-small-cell lung cancer
NSTEMI	non-ST-segment elevation myocardial infarction
NT-proBNP	N-terminal-proBNP
NTM	nontuberculous mycobacteria
NYHA	New York Heart Association
OA	osteoarthritis
OAT	organic anion transport
OATP1	organic anion-transporting polypeptide 1
OATP2	organic anion-transporting polypeptide 2
OCT	organic cation transport
OGTT	oral glucose tolerance test
OSHA	Occupational Safety and Health Administration
$\mathrm{P_1G_1O_1}$	one live birth, one pregnancy, no spontaneous or elective abortions
P-gp	P-glycoprotein
Pa	Pascal
pAB	polyclonal antibody
$\mathrm{PaCO_2}$	partial pressure of carbon dioxide, arterial
PAD	peripheral arterial disease
PAE	postantibiotic effect
PAI1	plasminogen activator inhibitor 1
pANCA	perinuclear antineutrophil cytoplasmic antibody
$\mathrm{PaO_2}$	partial pressure of oxygen, arterial
PAS	periodic acid-Schiff
PBC	primary biliary cirrhosis
PBMC	peripheral blood mononuclear cell
PBP	penicillin-binding protein
$\mathrm{PC_{20}FEV_1}$	provocation concentration of the bronchoconstrictor agent that produces a 20% reduction in $\mathrm{FEV_1}$
PCA	postconceptional age
PCI	percutaneous coronary intervention
$\mathrm{pCO_2}$	partial pressure of carbon dioxide
PCOS	polycystic ovary syndrome
PCP	phencyclidine
PCR	polymerase chain reaction
PCSK9	proprotein convertase subtilisin/kexin type 9
PD	pharmacodynamic
PDA	potato dextrose agar
PE	phycoerythrin
$\mathrm{Peak_{steady\ state}}$	Peak concentration of a drug in serum or plasma
PEA	phenylethyl alcohol
PEFR	peak expiratory flow rate
PET	positron emission tomography
PF3	platelet factor 3
PF4	platelet factor 4
PFA	potato flake agar
PFGE	pulsed-field gel electrophoresis
PFT	pulmonary function test
pg	picogram
PG	prostaglandin
PG2	prostacyclin
pH	power of hydrogen or hydrogen ion concentration
PHY	phenytoin

Ph	Philadelphia
PICU	pediatric intensive care unit
PID	pelvic inflammatory disease
PIP	proximal interphalangeal
PK	pharmacokinetic
PKU	phenylketonuria
PL	phospholipid
PMA	postmenstrual age
PMN	polymorphonuclear leukocyte
PNA	postnatal age
PNA-FISH	peptide nucleic acid fluorescent in situ hybridization
PO	per os (by mouth)
pO_2	partial pressure of oxygen
POC	point-of-care
POCT	point-of-care testing
PPAR	peroxisome proliferator-activated receptor
PPD	purified protein derivative
PPG	postprandial glucose
PPI	proton pump inhibitor
PR	progesterone receptor
PR3	proteinase 3
PRN	as needed
PRU	P2Y12 reaction units
PSA	prostate specific antigen
PSAD	prostate specific antigen density
PSB	protected specimen brush
PSM	patient self-management
PST	patient self-testing
PT	prothrombin time
PTCA	percutaneous transluminal coronary angioplasty
PTH	parathyroid hormone
q	every
Q	perfusion
QC	quality control
QID	four times daily
qPCR	real-time polymerase chain reaction
QRS	electrocardiograph wave; represents ventricular depolarization
QwMI	Q-wave myocardial infarction
R	resistant
R-CVA	right cerebral vascular accident
RA	rheumatoid arthritis
RAAS	renin-angiotensin-aldosterone system
RADT	rapid antigen detection test
RAEB	refractory anemia with excess blasts
RAIU	radioactive iodine uptake test
RALS	right-angle light scattering
RBC	red blood cell
RBF	renal blood flow
RCA	right coronary artery
RDW	red cell distribution width
RF	rheumatoid factor
RhMK	rhesus monkey kidney
RI	reticulocyte index
RIA	radioimmunoassay
RIBA	recombinant immunoblot assay
RIDTs	rapid influenza diagnostic tests
RNA	ribonucleic acid
RNP	ribonucleoprotein
Ro/SSA	Ro/Sjögren syndrome A antibody
RPF	renal plasma flow
RPR	rapid plasma reagin
RR	respiratory rate
RSA	rapid sporulation agar
RSAT	rapid streptococcal antigen test
RSV	respiratory syncytial virus
RT	reverse transcriptase; reverse transcription
RT-PCR	reverse-transcriptase polymerase chain reaction
RV	residual volume
S	susceptible
S Cys C	serum cystatin C
S:P ratio	saliva:plasma concentration ratio
SA	sinoatrial
SaO_2	arterial oxygen saturation
SAMHSA	Substance Abuse and Mental Health Services Administration
SAT	serum agglutination test
SBA	sheep blood agar
SBT	serum bactericidal test
Scl_{70}	scleroderma-70 or DNA topoisomerase I antibody
SCr	serum creatinine
$ScvO_2$	central venous oxygen saturation
SD	standard deviation
SDA	Sabouraud dextrose agar
SDA	strand displacement amplification
sec	second
SEGA	subependymal giant cell astrocytoma
SGE	spiral gradient endpoint
SGLT	sodium glucose cotransporters
SHBG	sex hormone-binding globulin
SI	International System of Units
SIADH	syndrome of inappropriate antidiuretic hormone
SID	strong iron difference
SIG	strong ion gap
SLE	systemic lupus erythematosus
Sm	Smith antibody
SMBG	self-monitoring blood glucose
SNP	single nucleotide polymorphism
SNRI	serotonin–norepinephrine reuptake inhibitor
SnRNP	small nuclear ribonucleoprotein particle
SPECT	single-photon emission computed tomography
SPEP	serum protein electrophoresis
SRA	C-serotonin release assay

ssDNA	single-stranded DNA
SSRI	selective serotonin reuptake inhibitor
STD	sexually transmitted disease
STEMI	ST segment elevation myocardial infarction
SV	stroke volume
SVC	slow vital capacity
SvO_2	venous oxygen saturation
T_3	triiodothyronine
T_3RU	triiodothyronine resin uptake
T_4	thyroxine
TAT	turnaround time
TB	tuberculosis
TBG	thyroxine-binding globulin
TBI	total body irradiation
TBPA	thyroid-binding prealbumin
TBW	total body water
TBW	total body weight
TC	total cholesterol
TCA	tricyclic antidepressant
TDM	therapeutic drug monitoring
TEE	transesophageal echocardiography
TF	tissue factor
TFPI	tissue factor pathway inhibitor
TG	triglyceride
THC	total hemolytic complement
TIA	transient ischemic attack
TIBC	total iron-binding capacity
TID	three times daily
TJC	The Joint Commission
TK	tyrosine kinase
TKI	tyrosine kinase inhibitor
TLA	total laboratory automation
TLC	therapeutic lifestyle changes
TLC	thin layer chromatography
TLC	total lung capacity
TMA	transcription mediated amplification
TN	true negative
TnC	troponin C
TNF	tumor necrosis factor
TnI	troponin I
TnT	troponin T
TP	true positive; tube precipitin
tPA	tissue plasminogen activator
TPMT	thiopurine methyltransferase
TPN	total parenteral nutrition
TR	therapeutic range
TRH	thyrotropin-releasing hormone
TRUS	transrectal ultrasound of the prostate
TSB	trypticase soy broth
TSH	thyroid-stimulating hormone
TST	tuberculin skin test
TT	thrombin time
TTE	transthoracic echocardiography
TTP	thrombotic thrombocytopenic purpura; total testing process
TTR	time in therapeutic range
TV	tidal volume
T_XA_2	thromboxane A_2
type 1 DM	type 1 diabetes mellitus
type 2 DM	type 2 diabetes mellitus
U	urinary creatinine concentration
U_1RNP	uridine-rich ribonuclear protein
UA	unstable angina
UCr	urine creatinine
UFC	urine-free cortisol
UFH	unfractionated heparin
UGT1A1	uridine diphosphate glucuronyl transferase
UKPDS	United Kingdom Prospective Diabetes Study
ULN	upper limit of normal
uNGAL	urine neutrophil gelatinase associated lipocalcin
uPA	urokinase plasminogen activator
UTI	urinary tract infection
UV	ultraviolet
V	total urine volume collected; ventilation; volt
VAP	ventilator-associated pneumonia
VC	vital capacity
Vd	volume of distribution
VDRL	Venereal Disease Research Laboratory
VISA	vancomycin-intermediate *Staphylococcus aureus*
VKORC1	vitamin K epoxide reductase complex subunit 1
VLDL	very low-density lipoprotein
V_{max}	maximum rate of metabolism
VPA	valproic acid
VO_2	oxygen consumption
VRE	vancomycin-resistant enterococci
VTE	venous thromboembolism
vWF	von Willebrand factor
VZV	varicella zoster virus
W	watt
WB	western blot
WBC	white blood cell
WHO	World Health Organization
WNL	within normal limits
Wt	weight
WT	wild type
yr	year

PART I

BASIC CONCEPTS AND TEST INTERPRETATIONS

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1

DEFINITIONS AND CONCEPTS

Karen J. Tietze

OBJECTIVES

After completing this chapter, the reader should be able to

- Differentiate between accuracy and precision
- Distinguish between quantitative, qualitative, and semiqualitative laboratory tests
- Define reference range and identify factors that affect a reference range
- Differentiate between sensitivity and specificity, and calculate and assess these parameters
- Identify potential sources of laboratory errors and state the impact of these errors in the interpretation of laboratory tests
- Identify patient-specific factors that must be considered when assessing laboratory data
- Discuss the pros and cons of point-of-care and at-home laboratory testing
- Describe a rational approach to interpreting laboratory results

Laboratory testing is used to detect disease, guide treatment, monitor response to treatment, and monitor disease progression. However, it is an imperfect science. Laboratory testing may fail to identify abnormalities that are present (false negatives [FNs]) or identify abnormalities that are not present (false positives [FPs]). This chapter defines terms used to describe and differentiate laboratory tests and describes factors that must be considered when assessing and applying laboratory test results.

DEFINITIONS

Many terms are used to describe and differentiate laboratory test characteristics and results. The clinician should recognize and understand these terms before assessing and applying test results to individual patients.

Accuracy and Precision

Accuracy and *precision* are important laboratory quality control measures. Laboratories are expected to test analytes with accuracy and precision and to document the quality control procedures. Accuracy of a quantitative assay is usually measured in terms of analytical performance, which includes accuracy and precision. *Accuracy* is defined as the extent to which the mean measurement is close to the true value. A sample spiked with a known quantity of an analyte is measured repeatedly; the mean measurement is calculated. A highly accurate assay means that the repeated analyses produce a mean value that is the same as or very close to the known spiked quantity. Accuracy of a qualitative assay is calculated as the sum of the true positives (TPs) and true negatives (TNs) divided by the number of samples tested (accuracy = [(TP + TN) ÷ number of samples tested] × 100%). *Precision* refers to assay reproducibility (i.e., the agreement of results when the specimen is assayed many times). An assay with high precision means the methodology is consistently able to produce results in close agreement. The accuracy of those results is a separate issue.

Analyte

The *analyte* is the substance measured by the assay. Some substances, such as phenytoin and calcium, are bound extensively to proteins such as albumin. Although the unbound fraction elicits the physiological or pharmacological effect (bound substances are inactive), most routine assays measure the total substance (bound plus unbound). The free fraction may be assayable, but the assays are not routine. Therefore, the reference range for total and free substances may be quite different. For example, the reference range is 10–20 mcg/mL for total phenytoin, 1–2 mcg/mL for free phenytoin, 9.2–11 mg/dL for total serum calcium, and 4–4.8 mg/dL for free (also called *ionized*) calcium.

Some analytes exist in several forms and each has a different reference range. These forms are referred to as *fractions, subtypes, subforms, isoenzymes*, or *isoforms*.

Note: This chapter is based, in part, on the second edition chapter titled "Definitions and Concepts" by Scott L. Traub.

Results for the total and each form are reported. For example, bilirubin circulates in conjugated and unconjugated subforms as well as bound irreversibly to albumin (delta bilirubin). *Direct bilirubin* refers to the sum of the conjugated plus the delta forms (water soluble forms); *indirect bilirubin* refers to the unconjugated form (water insoluble form). Lactate dehydrogenase (LDH) is separated electrophoretically into five different isoenzymes: LDH1, LDH2, LDH3, LDH4, and LDH5. Creatine kinase (CK) exists in three isoforms: CK-BB (CK1), CK-MB (CK2), and CK-MM (CK3).

Biomarker

A *biomarker* (biological marker) is a marker (not necessarily a quantifiable laboratory parameter) defined by the National Institutes of Health as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention."[1] Biomarkers are used to diagnose and stage disease (i.e., determine the extent of disease), assess disease progression, and predict or assess response to therapeutic interventions. Tumor markers are biomarkers used to identify the presence of some cancers, to stage disease, or to assess patient response to drug and nondrug cancer treatments. Many biomarkers are common laboratory parameters. For example, glycated hemoglobin A1c (HbA1c) is used to assess long-term glucose control in patients with diabetes.

Noninvasive Versus Invasive Tests

A *noninvasive test* is a procedure that examines fluids or other substances (e.g., urine and exhaled air) obtained without using a needle, tube, device, or scope to penetrate the skin or enter the body. An *invasive test* is a procedure that examines fluids or tissues (e.g., venous blood and skin biopsy) obtained by using a needle, tube, device, or scope to penetrate the skin or enter the body. Invasive tests pose variable risk depending on the method of specimen collection (e.g., pain and bruising associated with venipuncture) and are less convenient than noninvasive tests.

Predictive Value

The *predictive value*, derived from a test's sensitivity, specificity, and prevalence (incidence) of the disease in the population being tested, is used to assess a test's reliability (**Table 1-1**). As applied to a positive test result, the predictive value indicates the percent of positives that are TPs. For a test with equal sensitivity and specificity, the predictive value of a positive result increases as the incidence of the disease in the population increases. For example, the glucose tolerance test has a higher predictive value for diabetes in women who are pregnant than in the general population. A borderline abnormal serum creatinine (SCr) concentration has a higher predictive value for kidney disease in patients in a nephrology unit than in patients in a general medical unit. The lower the prevalence of disease in the population tested, the greater the chance that a positive test result is in error. The predictive value may also be applied to negative results. As applied to a negative test result, the predictive value indicates the percent of negatives that are TNs (**Minicase 1**).

TABLE 1-1. Relationship of Sensitivity, Specificity, Disease Prevalence, and Predictive Value of Positive Test[a,b]

SENSITIVITY AND SPECIFICITY (%)	PREVALENCE (%)	PREDICTIVE VALUE OF POSITIVE TEST (%)
95	0.1	1.9
	1	16.1
	2	27.9
	5	50
	50	95
99	0.1	9
	1	50
	2	66.9
	5	83.9
	50	99

[a] The predictive value of a positive test increases as the disease prevalence and sensitivity and specificity of the test increase.

[b] Predictive value of positive test = [TP ÷ (TP + FP)] x 100%. Predictive value of negative test = [TN ÷ (TN + FN)] x 100%. Disease prevalence = (TP + FN) ÷ number of patients tested. FN = diseased persons not detected by test (false negatives); FP = nondiseased persons positive to test (false positives); TN = nondiseased persons negative to test (true negatives); and TP = diseased persons detected by test (true positives).

Qualitative Tests

A *qualitative test* is a test whose results are reported as either positive or negative without further characterization of the degree of positivity or negativity. Exact quantities may be measured in the laboratory but are still reported qualitatively using predetermined ranges. For example, a serum or urine pregnancy test is reported as either positive or negative; a bacterial wound culture is reported as either positive for one or more specific microorganisms or reported as no growth; a urine toxicology drug screen is reported as either positive or negative for specific drugs; a hepatitis C viral ribonucleic acid (RNA) test is reported as positive or negative for hepatitis C viral RNA; and an acid-fast stain for *Mycobacterium* is reported as either positive or negative.

Quantitative Tests

A *quantitative test* is a test whose results are reported as an exact numeric measurement (usually a specific mass per unit measurement) and assessed in the context of a reference range of values. For example, serum potassium is reported in milliequivalents per liter, creatinine clearance (CrCl) is reported in milliliters per minute, and LDH is reported in units per liter. Some test results are reported as titers (dilutions). A serum antinuclear antibody titer of 1:160 is usually associated with active systemic lupus erythematosus or other autoimmune diseases, although some patients may have "low titer" disease with titers of 1:40 or 1:80.

MINICASE 1

Rapid Streptococcal Antigen Test

In 453 patients with acute pharyngitis symptoms, detection of group A β-hemolytic streptococci with a commercial rapid antigen detection test and standard throat culture are compared.[2] The package insert for the rapid streptococcal antigen test (RSAT) notes a sensitivity of 95% and a specificity of 98% when used according to the manufacturer instructions.

QUESTION: After reviewing the following results, what conclusions can be made about the clinical performance of the RSAT?

RSAT Results (*n* = 453):

True Positives	51	True Negatives	362
False Positives	12	False Negatives	28

DISCUSSION: Calculate sensitivity, specificity, predictive value of a positive test, and the predictive value of a negative test.

Sensitivity = (TP ÷ [TP + FN]) × 100% = (51 ÷ [51 + 28]) × 100% = 64.6%

Specificity = (TN ÷ [TN + FP]) × 100% = (362 ÷ [362 + 12]) × 100% = 96.8%

Predictive value of positive test = (TP ÷ [TP + FP]) × 100% = (51 ÷ [51 + 12]) × 100% = 81%

Predictive value of negative test = (TN ÷ [TN + FN]) × 100% = (362 ÷ [362 + 28]) × 100% = 92.8%

In this study, RSAT has a lower specificity and sensitivity than reported by the manufacturer; the sensitivity depends on proper throat swab collection. Appropriate healthcare training is important to achieve and maintain maximum sensitivity and positive predictive value of the test.

Reference Range

The *reference range* (also known as the *reference interval* or the *reference value*) is a statistically-derived numerical range obtained by testing a sample of individuals assumed to be healthy. The upper and lower limits of the range are not absolute (i.e., normal versus abnormal) but rather points beyond which the probability of clinical significance begins to increase. The term *reference range* is preferred over the term *normal range*.[3] The reference population is assumed to have a Gaussian distribution with 68% of the values within one standard deviation (SD) above and below the mean, 95% within ±2 SD, and 99.7% within ±3 SD (**Figure 1-1**).

The reference range for a given analyte is usually established in the clinical laboratory as the mean or average value plus or minus two SDs. Acceptance of the mean ±2 SD indicates that one in 20 normal individuals will have test results outside the reference range (2.5% have values below the lower limit of the reference range, and 2.5% have values above the upper limit of the reference range). Accepting a wider range (e.g., ±3 SD) includes a larger percentage (99.7%) of normal individuals but increases the chance of including individuals with values only slightly outside of a more narrow range, thus decreasing the sensitivity of the test.

Qualitative laboratory tests are either negative or positive and without a reference range; any positivity is considered abnormal. For example, any amount of serum acetone, porphobilinogen, or alcohol in serum or plasma is considered abnormal. The presence of glucose, ketones, blood, bile, or nitrate in urine is also abnormal. The results of the VDRL (Veneral Disease Research Laboratory) test, tests for red blood cell (RBC) sickling, and the malaria smear are either positive or negative.

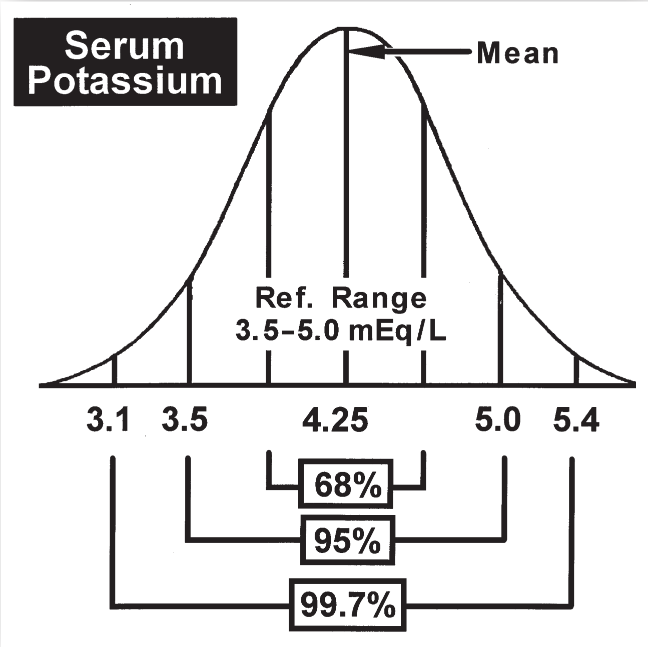

FIGURE 1-1. Gaussian (random) value distribution with a visual display of the area included within increments of standard deviation (SD) above and below the mean: ±1 SD = 68% of total values; ±2 SD = 95% of total values; and ±3 SD = 99.7% of total values.

Factors That Influence the Reference Range

Many factors influence the reference range. Reference ranges may differ between labs depending on analytical technique, reagent, and equipment. The initial assumption that the sample population is normal may be false. For example, the reference range is inaccurate if too many individuals with covert disease (i.e., no signs or symptoms of disease) are included in the sample population. Failure to control for physiologic variables (e.g., age, gender, ethnicity, body mass, diet, posture, and time of day) introduces many unrelated factors and may result in an inaccurate reference range. Reference ranges calculated from nonrandomly distributed (non-Gaussian) test results or from a small number of samples may not be accurate.

Reference ranges may change as new information relating to disease and treatments becomes available. For example, the National Cholesterol Education Program's 2002 Third Report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) lowered and more closely spaced reference range cutoff points for low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TGs) and recommended dose-adjusted drug therapy to achieve specific cholesterol goals.[4] Based on newer evidence, the 2013 American College of Cardiology/American Heart Association Guideline on the Treatment of Blood Cholesterol to Reduce Atherosclerotic Cardiovascular Risk in Adults does not recommend specific LDL-C treatment targets.[5] The generally accepted upper limit of normal (ULN) for thyroid-stimulating hormone (TSH) (4.12 mIU/L) is based on data from the National Health and Nutrition Examination Survey.[6] But the availability of more sensitive assays and the recognition that the original reference population data were skewed has led some clinicians to conclude that the ULN for TSH should be lowered.[7]

Critical Value

The term *critical value* refers to a result that is far enough outside the reference range that it indicates impending morbidity (e.g., potassium <2.8 mEq/L). Because laboratory personnel are not in a position to consider mitigating circumstances, a responsible member of the healthcare team is notified immediately on discovery of a critical value test result. Critical values may not always be clinically relevant because the reference range varies for the reasons discussed above.

Semiquantitative Tests

A *semiquantitative test* is a test whose results are reported as either negative or with varying degrees of positivity but without exact quantification. For example, urine glucose and urine ketones are reported as negative or 1+, 2+, 3+; the higher numbers represent a greater amount of the measured substance in the urine but not a specific concentration.

Sensitivity

The *sensitivity* of a test refers to the ability of the test to identify positive results in patients who actually have the disease (TP rate).[8,9] Sensitivity assesses the proportion of TPs disclosed by the test (**Table 1-2**). A test is completely sensitive (100% sensitivity) if it is positive in every patient who actually has the disease. The higher the test sensitivity, the lower the chance of a false-negative result; the lower the test sensitivity, the higher the chance of a false-negative result. However, a highly sensitive test is not necessarily a highly specific test (see below).

TABLE 1-2. Calculation of Sensitivity and Specificity[a]

SCREENING TEST RESULT	DISEASED	NOT DISEASED	TOTAL
Positive	TP	FP	TP + FP
Negative	FN	TN	FN + TN
Total	TP + FN	FP + TN	TP + FP + FN + TN

FN = diseased persons not detected by test (false negatives); FP = nondiseased persons positive to test (false positives); TN = nondiseased persons negative to test (true negatives); TP = diseased persons detected by test (true positives).

[a] Sensitivity = [TP ÷ (TP + FN)] x 100%. Specificity = [TN ÷ (TN + FP)] x 100%.

Highly sensitive tests are preferred when the consequences of not identifying the disease are serious; less sensitive tests may be acceptable if the consequence of an FN is less significant or if low sensitivity tests are combined with other tests. For example, inherited phenylalanine hydroxylase deficiency (phenylketonuria [PKU]) results in increased phenylalanine concentrations. High phenylalanine concentrations damage the central nervous system and are associated with mental retardation. Mental retardation is preventable if PKU is diagnosed and dietary interventions initiated before 30 days of age. The phenylalanine blood screening test, used to screen newborns for PKU, is a highly sensitive test when testing infants at least 24 hours of age.[10] In contrast, the prostate-specific antigen (PSA) test, a test commonly used to screen men for prostate cancer, is highly specific but has low sensitivity, especially at low PSA cutoff values of 4–10 ng/mL.[11] Thus, PSA cannot be relied on as the sole prostate cancer screening method.

Sensitivity also refers to the range over which a quantitative assay can accurately measure the analyte. In this context, a sensitive test is one that can measure low levels of the substance; an insensitive test cannot measure low levels of the substance accurately. For example, a digoxin assay with low sensitivity might measure digoxin concentrations as low as 0.7 ng/mL. Concentrations below 0.7 ng/mL would not be measurable and would be reported as <0.7 ng/mL whether the digoxin concentration was 0.69 ng/mL or 0.1 ng/mL. Therefore, this relatively insensitive digoxin assay would not differentiate between medication nonadherence with an expected digoxin concentration of 0 ng/mL and low concentrations associated with inadequate dosage regimens.

Specificity

Specificity refers to the percent of negative results in people without the disease (TN rate).[8,9] Specificity assesses the proportion of TNs disclosed by the test (Table 1-2); the lower the specificity, the higher the chance of a false-positive result. A test with a specificity of 95% for the disease in question indicates that the disease will be detected in 5% of people without the disease. Tests with high specificity are best for confirming a diagnosis because the tests are rarely positive in the absence of the disease. Several newborn screening tests (e.g., PKU, galactosemia, biotinidase deficiency, congenital hypothyroidism, and congenital adrenal hyperplasia) have specificity levels above 99%.[12] In contrast, the erythrocyte sedimentation rate (ESR) is a nonspecific test; infection, inflammation, and plasma cell dyscrasias increase the ESR.

Specificity as applied to quantitative laboratory tests refers to the degree of cross-reactivity of the analyte with other substances in the sample. Quinine may cross react with or be measured as quinidine in some assays, falsely elevating reported quinidine concentrations. Phenazopyridine interferes with urine ketone tests using sodium nitroprusside (e.g., Ketostix).

Specimen

A *specimen* is a sample (e.g., whole blood, plasma, serum, urine, stool, sputum, sweat, gastric secretions, exhaled air, cerebrospinal fluid, or tissues) that is used for laboratory analysis. Plasma is the watery acellular portion of blood. Plasma contains dissolved proteins (e.g., albumin, globulins, fibrinogen, enzymes, and hormones), electrolytes (e.g., sodium, potassium, chloride, calcium, and magnesium), lipids, carbohydrates, amino acids, and other organic substances (e.g., urea, uric acid, creatinine, bilirubin, ammonium ions). Serum is the liquid that remains after the fibrin clot is removed from plasma. Although some laboratory tests are performed only on plasma (e.g., prothrombin time, activated partial thromboplastin time [aPTT], D-dimer, and fibrinogen concentrations) or serum (e.g., albumin, creatinine, bilirubin, and acetaminophen concentrations), other laboratory tests can be performed on either plasma or serum (e.g., glucose, cortisol, electrolytes, and phenytoin concentrations). Some tests are performed on whole blood (e.g., blood gases, hemoglobin, hematocrit, complete blood count [CBC], and ESR).

LABORATORY TEST RESULTS

Units Used in Reporting Laboratory Results

Laboratory test results are reported with a variety of units. For example, four different units are used to report serum magnesium concentration (1 mEq/L = 1.22 mg/dL = 0.5 mmol/L = 12.2 mg/L). Additionally, the same units may be reported in different ways. For example, mg/dL, mg/100 mL, and mg% are equivalent units. Enzyme activity is usually reported in terms of units, but the magnitude varies widely and depends on the methodology. Rates are usually reported in volume per unit of time (e.g., CrCl is measured in mL/min or L/hr), but the ESR is reported in mm/hr and coagulation test results are reported in seconds or minutes. This lack of standardization is confusing and may lead to misinterpretation of the test results.

The International System of Units (Système Internationale d'Unités, or SI) was created about 50 years ago to standardize quantitative units worldwide.[13] Four base units and symbols are designated: length (meter, m), mass (kilogram, kg), time (second, s), and substance (mole, mol). Five derived units are designated: volume (liter, L, 10^{-3} m^3), force (newton, N, kg ms^{-2}), pressure (pascal, Pa, kg m^{-1} s^{-2}), energy (joule, J, kg m^2 s^{-2}), and power (watt, W, kg m^2 s^{-3}). However, it is difficult for clinicians to relate to molar concentrations (e.g., serum cholesterol 4.14 mmol × L^{-1} versus 160 mg/dL, or HbA1c mmol/mL versus 8%). In the United States, most laboratory results are reported in conventional units.

Rationale for Ordering Laboratory Tests

Laboratory tests are performed with the expectation that the results will

- discover occult disease
- confirm a suspected diagnosis
- differentiate among possible diagnoses
- determine the stage, activity, or severity of disease
- detect disease recurrence
- assess the effectiveness of therapy
- guide the course of therapy

Laboratory tests are categorized as screening or diagnostic tests. Screening tests, performed in individuals without signs or symptoms of disease, detect disease early when interventions (e.g., lifestyle modifications, drug therapy, and surgery) are likely to be effective. Screening tests are performed on healthy individuals and are generally inexpensive, quick and easy to perform, and reliable, although they do not provide a definitive answer. Screening tests require confirmation with other clinical tests. Diagnostic tests are performed on at-risk individuals, are typically more expensive, and are associated with some degree of risk but provide a definitive answer.[14]

Comparative features of screening tests are listed in **Table 1-3**. Examples of screening tests include the Papanicolaou smear, lipid profile, PSA, fecal occult blood, tuberculin skin test, sickle cell tests, blood coagulation tests, and serum chemistries. Screening tests may be performed on healthy outpatients (e.g., ordered by the patient's primary care provider or performed during public health fairs) or on admission to an acute care facility (e.g., prior to scheduled surgery). Abnormalities identified during screening are followed by more specific tests to confirm the results.

TABLE 1-3. Comparative Features of Screening and Diagnostic Laboratory Tests

FEATURE	SCREENING TEST	DIAGNOSTIC TEST
Simplicity of test	Fairly simple	More complex
Target population	Individuals without signs or symptoms of the disease	Individuals with signs or symptoms of the disease
Characteristic	High sensitivity	High specificity
Disease prevalence	Relatively common	Common or rare
Risks	Acceptable to population	Acceptable to individual

Source: Reference 15.

Screening tests must be cost-effective and population-appropriate. The number needed to screen is defined as "the number of people that need to be screened for a given duration to prevent one death or one adverse event."[16] For example, 84 women between the ages of 40 and 84 years need to undergo annual mammographic screening to prevent one death from breast cancer.[17]

Diagnostic tests are performed in individuals with signs or symptoms of disease, a history suggestive of a specific disease or disorder, or an abnormal screening test. Diagnostic tests are used to confirm a suspected diagnosis, differentiate among possible diagnoses, determine the stage of activity of disease, detect disease recurrence, and assess and guide the therapeutic course. Diagnostic test features are listed in Table 1-3. Examples of diagnostic tests include blood cultures, serum cardiac-specific troponin I and T, kidney biopsy, and the cosyntropin test.

Many laboratories group a series of related tests (screening and/or diagnostic) into a set called a *profile*. For example, the basic metabolic panel (BMP) includes common serum electrolytes (sodium, potassium, and chloride), carbon dioxide content, blood urea nitrogen (BUN), calcium, creatinine, and glucose. The comprehensive metabolic panel includes the BMP plus albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, and total protein. Grouped together for convenience, some profiles may be less costly to perform than the sum of the cost of each individual test. However, profiles may generate unnecessary patient data. Attention to cost is especially important in the current cost-conscious era. A test should not be done if it is unnecessary, redundant, or provides suboptimal clinical data (e.g., non-steady-state serum drug concentrations). Before ordering a test, the clinician should consider the following questions:

- Was the test recently performed and in all probability the results have not changed at this time?
- Were other tests performed that provide the same information?
- Can the information be estimated with adequate reliability from existing data?

  (For example, CrCl can be estimated using age, height, weight, and SCr rather than measured from a 24-hour urine collection. Serum osmolality can be calculated from electrolytes and glucose rather than measured directly.)
- What will I do if results are positive or negative (or absent or normal)? (For example, if the test result will not aid in clinical decisions or change the diagnosis, prognosis, or treatment course, the benefits from the test are not worth the cost of the test.)

Factors That Influence Laboratory Test Results

Laboratory results may be inconsistent with patient signs, symptoms, or clinical status. Before accepting reported laboratory values, clinicians should consider the numerous laboratory-specific and patient-specific factors that may influence the results (**Table 1-4**). For most of the major tests discussed in this book, a Quickview chart summarizes information helpful in interpreting results. **Figure 1-2** depicts the format and content of a typical Quickview chart.

TABLE 1-4. Factors That Influence Assessment of Laboratory Results

Assay used and form of analyte
Free form
Bound form
Clinical situation
Acuity of disease
Severity of disease
Demographics
Age
Gender
Ethnicity
Height
Weight
Body surface area
Drugs
Drug–drug interactions
Drug–assay interactions
Food
Time of last meal
Type of food ingested
Nutritional status
Well nourished
Poorly nourished
Posture
Upright
Supine
Pregnancy
Specimen analyzed
Serum
Plasma
Whole blood (venous or arterial)
Cerebrospinal fluid
Urine
Stool
Sputum
Other (e.g., tissue, sweat, gastric contents, effusions)
Temporal relationships
Time of day
Time of last dose

QUICKVIEW | Contents of a Typical Quickview Chart

PARAMETER	DESCRIPTION	COMMENTS
Common reference ranges		
Adults	Reference range in adults	Variability and factors affecting range
Pediatrics	Reference range in children	Variability, factors affecting range, age grouping
Critical value	Value beyond which immediate action usually needs to be taken	Disease-dependent factors; relative to reference range; value is a multiple of upper normal limit
Inherent activity	Does substance have any physiological activity?	Description of activity and factors affecting activity
Location		
Production	Is substance produced? If so, where?	Factors affecting production
Storage	Is substance stored? If so, where?	Factors affecting storage
Secretion/excretion	Is substance secreted/excreted? If so, where/how?	Factors affecting secretion or excretion
Causes of abnormal values		
High	Major causes	Modification of circumstances, other related causes or drugs that are commonly monitored with this test
Low	Major causes	
Signs and symptoms		
High level	Major signs and symptoms with a high or positive result	Modification of circumstances/other related signs and symptoms
Low level	Major signs and symptoms with a low result	Modification of circumstances/other related causes
After event, time to....		
Initial elevation	Minutes, hours, days, weeks	Assumes acute insult
Peak values	Minutes, hours, days, weeks	Assumes insult not yet removed
Normalization	Minutes, hours, days, weeks	Assumes insult removed and nonpermanent damage
Causes of spurious results	List of common causes	Modification of circumstances/assay specific
Additional information	Any other pertinent information regarding the laboratory value or assay	

FIGURE 1-2. Contents of a typical Quickview chart.

Laboratory-Specific Factors

Laboratory errors are uncommon but may occur. Defined as a test result that is not the true result, *laboratory error* most appropriately refers to inaccurate results that occur because of an error made by laboratory personnel or equipment. However, laboratory error is sometimes used to refer to otherwise accurate results rendered inaccurate by specimen-related issues. Laboratory errors should be suspected for one or more of the following situations:

- The result is inconsistent with trend in serial test results.
- The magnitude of error is great.
- The result is not in agreement with a confirmatory test result.
- The result is inconsistent with clinical signs or symptoms or other patient-specific information.

True laboratory errors (inaccurate results) are caused by one or more laboratory processing or equipment errors, such as deteriorated reagents, calibration errors, calculation errors, misreading the results, computer entry or other documentation errors, or improper sample preparation. For example, incorrect entry of thromboplastin activity (ISI [international sensitivity index]) when calculating the international normalized ratio (INR) results in accurately assayed but incorrectly reported INR results.

Accurate results may be rendered inaccurate by one or more specimen-related problems. Improper specimen handling prior to or during transport to the laboratory may alter analyte concentrations between the time the sample was obtained from the patient and the time the sample was analyzed in the laboratory.[18] For example, arterial blood withdrawn for blood gas analysis must be transported on ice to prevent continued in vitro changes in pH, $PaCO_2$, and PaO_2. Failure to remove the plasma or serum from the clot within four hours of obtaining blood for serum potassium analysis may elevate the reported serum potassium concentration. Red blood cell hemolysis elevates the serum potassium and phosphate concentrations. Failure to refrigerate samples may cause falsely low concentrations of serum enzymes (e.g., CK). Prolonged tourniquet time may hemoconcentrate analytes, especially those that are highly protein bound (e.g., calcium).

Patient-Specific Factors

Laboratory test values cannot be interpreted in isolation of the patient. Numerous age-related (e.g., decreased renal function) and other patient-specific factors (e.g., time of day, posture) as well as disease-specific factors (e.g., time course) affect laboratory results. The astute clinician assesses laboratory data in context of all that is known about the patient.

Time course. Incorrectly timed laboratory tests produce misleading laboratory results. Disease states, normal physiologic patterns, pharmacodynamics, and pharmacokinetics time courses must be considered when interpreting laboratory values. For example, digoxin has a prolonged distribution phase. Digoxin serum concentrations obtained before tissue distribution is complete do not accurately reflect true tissue drug concentrations. Postmyocardial infarction enzyme patterns are complex and evolve over a prolonged period of time. For example, CK increases about six hours following myocardial infarction (MI) and returns to baseline about 48–72 hours after the MI. Following MI, LDH increases about 12–24 hours following MI and returns to baseline about 10 days after the MI. Troponin increases a few hours following MI and returns to baseline in about five to seven days. Serial samples are used to assess myocardial damage.

Laboratory samples obtained too early or too late may miss critical changes and lead to incorrect assessments. For example, cosyntropin (synthetic adrenocorticotropic hormone [ACTH]) tests adrenal gland responsiveness. The baseline 8 a.m. plasma cortisol is compared to the stimulated plasma cortisol obtained 30 and 60 minutes following injection of the drug. Incorrect timing leads to incorrect results. The sputum acid-fast bacilli (AFB) smear may become AFB-negative with just a few doses of antituberculous drugs, but the sputum culture may remain positive for several weeks. Expectations of a negative sputum culture too early in the time course may lead to the inappropriate addition of unnecessary antituberculous drugs.

Non-steady-state drug concentrations are difficult to interpret; inappropriate dosage adjustments (usually inappropriate dosage increases) may occur if the clinician fails to recognize that a drug has not reached steady-state concentrations. Although non-steady-state drug concentrations may be useful when assessing possible drug toxicity (e.g., overdose situations and new onset adverse drug events), all results need to be interpreted in the context of the drug's pharmacokinetics. Absorption, distribution, and elimination may change with changing physiology. For example, increased/decreased hepatic or renal perfusion may affect the clearance of a drug. Some drugs (e.g., phenytoin) have very long half-lives; constantly changing hemodynamics during an acute care hospitalization may prevent the drug from achieving steady state while the patient is acutely ill.

Age. Age influences many physiologic systems. Age-related changes are well-described for neonates and young children, but less data are available for the elderly and the very elderly (usually described as ≥90 years of age). Age influences some but not all laboratory values; not all changes are clinically significant.

Pediatric reference ranges often reflect physiologic immaturity, with laboratory values approaching those of healthy adults with increasing age. For example, the CBC (hemoglobin, hematocrit, RBC count, and RBC indices) ranges are greatly dependent on age with different values reported for premature neonates, term neonates, and young children. The fasting blood glucose reference range in premature neonates is approximately 20–65 mg/dL compared to 60–105 mg/dL for children two years of age and older and 70–110 mg/dL for adults. The SCr reference range for children 1–5 years of age differs from the reference range for children 5–10 years of age (0.3–0.5 mg/dL versus 0.5–0.8 mg/dL). Reference ranges for children are well-described because it is relatively easy to identify age-differentiated populations of healthy children. Most laboratory reference texts provide age-specific reference values.

Geriatric reference ranges are more difficult to establish because of physiologic variability with increasing age and the presence of symptomatic and asymptomatic disease states that influence reference values. Diet (e.g., malnutrition) also influences some laboratory results. Some physiologic functions (e.g., cardiac, pulmonary, renal and metabolic functions) progressively decline with age, but each organ declines at a different rate.[19] Other physiologic changes associated with aging include decreased body weight, decreased height, decreased total body water, increased extracellular water, increased fat percentage, and decreased lean tissue percentage; and loss of cell membranes integrity.[19] Published studies sometimes lead to contradictory conclusions due to differences in study methodology (e.g., single point versus longitudinal evaluations) and populations assessed (e.g., nursing home residents versus general population). Limited data are available for the very elderly (≥90 years of age).[20] Most laboratory reference texts provide age-specific reference values.

Despite the paucity of data and difficulties imposed by different study designs and study populations, there is general consensus that some laboratory reference ranges are unchanged, some are different but of uncertain clinical significance, and some are significantly different in the elderly (**Table 1-5**). For example, decreased lean muscle mass with increased age results in decreased creatinine production. Decreased renal function is associated with decreased creatinine elimination. Taken together, the SCr reference range in the elderly is not different from younger populations; however, CrCl clearly declines with age.

Significant age-related changes are reported for the two-hour postprandial glucose test, serum lipids, and arterial oxygen pressure (Table 1-5). The two-hour postprandial glucose increases by about 5–10 mg/dL per decade. Progressive ventilation-perfusion mismatching from loss of elastic recoil with increasing age causes progressively decreased arterial

TABLE 1-5. Laboratory Testing: Tests Affected by Aging

No change
Amylase
Lipase
Hemoglobin
Hematocrit
RBC count
RBC indices
Platelet count
WBC count and differential
Serum electrolytes (sodium, potassium, chloride, bicarbonate, magnesium)
Coagulation
Total iron-binding capacity
Thyroid function tests (thyroxine, T_3RU)
Liver function tests (AST, ALT, LDH)
Some change (unclear clinical significance)
Alkaline phosphatase
ESR
Serum albumin
Serum calcium
Serum uric acid
Thyroid function tests (TSH, T_3)
Clinically significant change
Arterial oxygen pressure
Two-hour postprandial glucose
Serum lipids (total cholesterol, LDL, TGs)
Serum testosterone (in men)
Serum estradiol (in women)
No change but clinically significant decrease in renal function
SCr

ALT = alanine aminotransferase; AST = aspartate aminotransferase; ESR = erythrocyte sedimentation rate; LDH = lactate dehydrogenase; LDL = low-density lipoprotein; TSH = thyroid-stimulating hormone; T_3 = triiodothyronine; T_3RU = triiodothyronine resin uptake; RBC = red blood cell; SCR = serum creatinine; TGs = triglycerides; WBC = white blood cell.
Source: References 19–27.

oxygen pressure with increasing age. Total cholesterol and LDL-C increase with age then decline in the very old.

Genetics, ethnicity, and gender. Inherited ethnic and/or gender differences are identified for some laboratory tests. For example, the hereditary anemias (e.g., thalassemias and sickling disorders such as sickle cell anemia) are more common in individuals with sub-Saharan African, Asian, Middle Eastern, and Mediterranean, ancestry.[28] Glucose-6-phosphate dehydrogenase (G6PD) deficiency is an example of an inherited sex-linked (X chromosome) enzyme deficiency found primarily in men of African, Asian, Middle Eastern, and Mediterranean ancestry.[29] The A-G6PD variant occurs mostly in Africans and affects about 13% of African-American males and 3% of African-American females in the United States. The Mediterranean G6PD variant, associated with a less common but more severe enzyme deficiency state, occurs mostly in individuals of Greek, Sardinian, Kurdish, Asian, and Sephardic Jewish ancestry.

Other enzyme polymorphisms influence drug metabolism. The genetically-linked absence of an enzyme may lead to drug toxicity secondary to drug accumulation or lack of drug effectiveness if the parent compound is an inactive prodrug (e.g., codeine). The cytochrome P450 (CYP450) superfamily consists of greater than 100 isoenzymes with selective but overlapping substrate specificity. Some individuals are poor metabolizers, while some are hyperextensive metabolizers. Several of the cytochrome P450 phenotypes vary by race. For example, the CYP2D6 poor metabolism phenotype occurs in 5–10% of Caucasians, and the CYP2C19 poor metabolism phenotype occurs in 10–30% of Asians.[30,31]

Additional enzyme polymorphisms include pseudocholinesterase deficiency, phenytoin hydroxylation deficiency, inefficient N-acetyltransferase activity, inefficient or rapid debrisoquine hydroxylase activity, diminished thiopurine methyltransferase activity, partial dihydropyrimidine dehydrogenase inactivity, and defective uridine diphosphate glucuronosyltransferase activity.[32] Other examples of genetic polymorphisms include variations in the β-2 adrenoceptor gene that influence response to sympathomimetic amines and variations in drug transporters such as P-glycoprotein, multidrug resistance-associated proteins (e.g., MRP1, MRP2, MRP3), and organic anion-transporting polypeptides (e.g., OATP1, OATP2).[32]

Biologic rhythms. Biologic rhythms are characterized as short (<30 minutes), intermediate (greater than 30 minutes but less than six days), and long (greater than six days).[33] The master clock, located in the suprachiasmatic nucleus of the hypothalamus, coordinates timing signals and multiple peripheral clocks.[34] A circadian rhythm is a 24-hour, endogenously generated cycle.[35] Well-described, human circadian rhythms include body temperature, cortisol production, melatonin production, and hormonal production (gonadotropin, testosterone, growth hormone, and thyrotropin). Platelet function, cardiac function, and cognition also follow a circadian rhythm.[36]

Other laboratory parameters follow circadian patterns. For example, statistically significant circadian rhythms have been reported for CK, ALT, γ-glutamyl transferase, LDH, and some serum lipids.[37,38] Glomerular filtration has a circadian rhythm.[39] Circadian variations in aminoglycoside pharmacokinetics, including netilmicin, amikacin, and gentamicin, have been reported.[40] Although the clinical significance of diurnally variable laboratory results is not well understood, diurnal variability should be considered when assessing laboratory values. Obtaining laboratory results at the same time of day (e.g., routine 7 a.m. blood draws) minimizes variability due to circadian rhythms. Different results obtained at different times of the day may be due to circadian variability rather than acute physiologic changes.

Other well-described biologic rhythms include the eight-hour rhythm for circulating endothelin, the approximately weekly (circaseptan) rhythm for urinary 17-ketosteroid excretion, the monthly rhythms of follicle-stimulating hormone [FSH], luteinizing hormone, progesterone production, and the seasonal rhythms for cholesterol and 25-hydroxycholecalciferol.[41,42]

Drugs. The four generally accepted categories of drug–laboratory interactions include methodological interference; drug-induced, end-organ damage; direct pharmacologic effect; and a miscellaneous category. Many drugs interfere with analytical methodology. Drugs that discolor the urine interfere with fluorometric, colorimetric, and photometric tests and mask abnormal urine colors. For example, amitriptyline turns the urine a blue-green color and phenazopyridine and rifampin turn the urine an orange-red color. Other drugs directly interfere with the laboratory assay. For example, high doses of ascorbic acid (>500 mg/day) cause false-negative stool occult blood tests. Some drugs interfere with urinary fluorescence tests for urine catecholamines by producing urinary fluorescence themselves (e.g., ampicillin, chloral hydrate, and erythromycin).

Direct drug-induced, end-organ damage (e.g., kidney, liver, and bone marrow) change the expected laboratory results. For example, amphotericin B causes renal damage evidenced by increased SCr. Bone marrow suppressants, such as doxorubicin and bleomycin, cause thrombocytopenia. Some drugs alter laboratory results as a consequence of a direct pharmacologic effect. Thiazide and loop diuretics increase serum uric acid by decreasing uric acid renal clearance or tubular secretion. Narcotics, such as codeine and morphine sulfate, increase serum lipase by inducing spasms of the sphincter of Oddi. Urinary specific gravity is increased in the presence of dextran. Other examples of drug–laboratory interactions include drugs that cause a positive direct Coombs test (e.g., isoniazid, sulfonamides, and quinidine), drugs that cause a positive antinuclear antibody test (e.g., penicillins, sulfonamides, and tetracyclines), and drugs that inhibit bacterial growth in blood or urine cultures (e.g., antibiotics).

Thyroid function tests are a good example of the complexity of potential drug-induced laboratory test changes. Thyroxine (T_4) and triiodothyronine (T_3) are displaced from binding proteins by salicylates, heparin, and high doses of furosemide. Free T_4 levels initially increase, but chronic drug administration results in decreased T_4 levels with normal TSH levels. Phenytoin, phenobarbital, rifampin, and carbamazepine stimulate hepatic metabolism of thyroid hormone, resulting in decreased serum hormone concentration. Amiodarone, high-dose β-adrenergic blocking drugs, glucocorticosteroids, and some iodine contrast dyes interfere with the conversion of T_4 to T_3. Ferrous sulfate, aluminum hydroxide, sucralfate, colestipol, and cholestyramine decrease T_4 absorption. Somatostatin, octreotide, and glucocorticosteroids suppress TSH production.

Pregnancy. Pregnancy is a normal physiologic condition that alters the reference range for many laboratory tests. Normal pregnancy increases serum hormone concentrations (e.g., estrogen, testosterone, progesterone, human chorionic gonadotropin, prolactin, corticotropin-releasing hormone, ACTH, cortisol, and atrial natriuretic hormone). The plasma volume increases by 30–50%, resulting in a relative hyponatremia (e.g., serum sodium decreased by about 5 mEq/L) and modest decreases in hematocrit. The metabolic adaptations to pregnancy include increased RBC mass and altered carbohydrate (e.g., 10–20% decrease in fasting blood glucose) and lipid (e.g., 300% increase in TGs and a 50% increase in total cholesterol) metabolism. Pregnancy changes the production and elimination of thyroid hormones, resulting in different reference values over the course of pregnancy.[43] For example, T_4-binding globulin increases during the first trimester, but pregnancy-associated accelerated thyroid hormone metabolism occurs later in the pregnancy. Other physiologic changes during pregnancy include an increased cardiac output (increases by 30–50%), decreased systemic vascular resistance, increased glomerular filtration rate (increases by 40–50%), shortened prothrombin and partial thromboplastin times, and hyperventilation resulting in compensated respiratory alkalosis and increased arterial oxygenation.[44]

Other Factors

Organ function, diet, fluid status, patient posture, and altitude also affect some laboratory tests.

Organ function. Renal dysfunction may lead to hyperkalemia, decreased CrCl, and hyperphosphatemia. Hepatic dysfunction may lead to reduced clotting factor production with prolonged partial thromboplastin times and prothrombin times. Bone marrow dysfunction may lead to pancytopenia.

Diet. Serum glucose and lipid profiles are best assessed in the fasting state. Unprocessed grapefruit juice down-regulates intestinal CYP3A4 and increases the bioavailability of some orally administered drugs.

Fluid status. Dehydration is associated with a decreased amount of fluid in the bloodstream; all blood constituents (e.g., sodium, potassium, creatinine, glucose, and BUN) become more concentrated. This effect is called *hemoconcentration*. Although the absolute amount of the substance in the body has not changed, the loss of fluid results in an abnormally high concentration of the measured analyte. The converse is true with hemodilution. Relativity must be applied or false impressions may arise (**Minicase 2**).

Posture. Plasma renin release is stimulated by upright posture, diuretics, and low-sodium diets; plasma renin testing usually occurs after two to four weeks of normal sodium diets under fasting supine conditions.

Altitude. At high altitude, hemoglobin initially increases secondary to dehydration. However, hypoxia stimulates erythropoietin production, which in turn stimulates hemoglobin production resulting in increased hemoglobin concentration and increased blood viscosity. Serum hemoglobin reference ranges are adjusted progressively upward for individuals living above 1000 feet.[45]

MINICASE 2

Interpretation of Laboratory Parameters in Dehydration

Jenny M., a 27-year-old female, was lost in the woods for several days without food or water. It was warm when the sun was out but cool at night. When rescued, she was happy to be found but was confused and weak. She had multiple scratches on her arms and legs and a deep gash across her cheek. Jenny M. was not taking any prescription or nonprescription medications.

On arrival in the emergency department, Jenny M. is weak, lethargic, and confused. Pertinent findings on physical examination include hypotension, tachycardia, tachypnea, and decreased skin turgor. Her mouth and lips are very dry. A BMP and CBC is ordered. The BMP was notable for elevated serum electrolytes, BUN, and SCr. The BUN-to-creatinine ratio is >20 to 1. The CBC is notable for an elevated white blood cell count with an increased percentage of bands and elevated platelets, hematocrit, and hemoglobin. Fluid resuscitation and antibiotics are ordered.

QUESTION: The prescribed antibiotic is dosed according to renal function. The manufacturer provides dosing recommendations based on estimated CrCl using the Cockcroft Gault equation. Is it appropriate to use this patient's admission creatinine to estimate her CrCl?

DISCUSSION: No. This patient is severely dehydrated; all laboratory parameters are hemoconcentrated. Her admission SCr is high and underestimates her renal function; however, it is possible that the severe dehydration damaged her kidneys. The best approach is to give one dose of the antibiotic and then monitor her renal function closely over the next 24–48 hours, redosing the antibiotic as indicated.

NONCENTRALIZED LABORATORY TESTS

Point-of-Care Testing

Point-of-care testing (POCT), also known as *near patient testing*, *bedside testing*, or *extra-laboratory testing*, is clinician-directed diagnostic testing performed at or near the site of patient care rather than in a centralized laboratory.[46,47] The POCT equipment ranges from small, hand-held devices to table-top analyzers. In vitro, in vivo, and ex vivo POCT refer to tests performed near the patient (e.g., fingerstick blood glucose), in the patient (e.g., specialized intra-arterial catheter that measures lactate), and just outside the patient (e.g., intra-arterial catheter attached to an external analyzer). Although POCT is not a new concept, recent technological advances (e.g., microcomputerization, miniaturization, biosensor development, etc.) have rapidly expanded the variety of available POCT beyond the traditional urinalysis dipsticks or fingerstick blood glucose monitors (**Table 1-6**).

The major advantages of POCT include reduced turnaround time and test portability. Reduced turnaround time is especially advantageous in settings where rapidly available laboratory test results may improve patient care (e.g., emergency departments, operating rooms, critical care units, accident scenes, and patient transport). Reduced turnaround time also enhances patient care in more traditional ambulatory settings by reducing patient and provider time and minimizing delays in initiating therapeutic interventions. Patient care sites without local access to centralized laboratories (e.g., nursing homes, rural physician practices, and military field operations) also benefit from POCT. Other POCT advantages include blood conservation (POCT usually require drops of blood as opposed to the several milliliters required for traditional testing); less chance of preanalytical error from inappropriate transport, storage, or labeling of samples; and overall cost savings. Although the per test cost is usually higher with POCT, cost analyses must consider the per unit cost of the test as well as other costs such as personnel time, length of stay, and quality of life.

The major disadvantages of POCT include misuse or misinterpretation of results, loss of centrally-generated epidemiological data, documentation errors, inappropriate test material disposal, and quality assurance issues. All laboratory testing must meet the minimum standards established by the Clinical Laboratory Improvement Amendments of 1988 (CLIA-88).[49] Under CLIA-88, tests are categorized into one of three groups based on potential public health risk: waived tests, tests of moderate complexity, and tests of high complexity. Waived tests (e.g., fecal occult blood test) pose no risk of harm to the patient if used incorrectly or use such simple and accurate methodologies that inaccurate results are unlikely. Many POCT meet the criteria for waived status but increasingly sophisticated POCT

TABLE 1-6. Common Point-of-Care Tests

Urine pregnancy test
Urine leukocytes or nitrite
Blood glucose
INR
Hemoglobin
Fecal occult blood
Throat swab for group A streptococci
C-reactive protein
Quantitative β-human chorionic gonadotropin
HbA1c
Nose/throat swab for influenza
Platelet count

HbA1c = glycated hemoglobin; INR = international normalized ratio.
Source: Reference 48.

TABLE 1-7. Types of Nonprescription In Vitro Diagnostic Tests

TEST	BODY FLUID OR SPECIMEN TESTED
Alcohol	Breath
Blood, fecal occult	Feces
Drugs of abuse (amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine metabolites, methadone, methylenedioxymethamphetamine, morphine, phencyclidine)	Urine, hair
Fertility, male	Semen
FSH (menopausal)	Urine
Glucose	Blood, urine
HDL cholesterol	Blood
Hemoglobin	Blood
HbA1c	Blood
HIV-1	Blood
Human chorionic gonadotropin (pregnancy)	Urine, serum
Ketones	Blood, urine
Luteinizing hormone (ovulation)	Urine
Thyroid-stimulating hormone	Blood
Triglycerides	Blood

FSH = follicle-stimulating hormone; HbA1c = glycated hemoglobin; HDL = high-density lipoprotein; HIV-1 = human immunodeficiency virus type 1.

may be subject to more stringent control. State-specific regulations may be more stringent than federal regulations.

Home Testing

Home testing refers to patient-directed diagnostic and monitoring testing usually performed by the patient or family member at home. More than 500 Food and Drug Administration (FDA)-approved, home-use, nonprescription laboratory test kits are marketed; home glucose and pregnancy testing are among the most popular (**Table 1-7**). Many non-FDA-approved home-testing kits are marketed via the Internet. The FDA's Office of In Vitro Diagnostics and Radiological Health maintains a searchable list of approved home-testing kits (http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/LabTest/ucm126079.htm). Advantages of home testing include convenience, cost savings (as compared to physician office visit), quickly available results, and privacy. Home monitoring of chronic drug therapy, such as blood glucose control with insulin therapy, may give the patient a better sense of control over the disease and improve patient outcomes. Disadvantages of home testing include misinterpretation of test results, delays in seeking medical advice, and lack of pretest and posttest counseling and psychological support. In addition, home test kits typically do not provide the consumer with information regarding sensitivity, specificity, precision, or accuracy. Home-use test kits are marketed as either complete test kits (consumers obtain their own sample, test the sample, and read the results) or as collection kits (consumers obtain the sample, mail the sample to the laboratory, and receive the results by mail or telephone). As always, consumers should read and follow the test instructions to minimize testing error.

GUIDELINES FOR INTERPRETING LABORATORY RESULTS

Laboratory results must be interpreted in context of the patient and the limitations of the laboratory test. However, a laboratory result is only one piece of information; diagnostic and therapeutic decisions cannot be made on the basis of one piece of information. Clinicians typically give more weight to the presence or absence of signs and symptoms associated with the medical problem rather than to an isolated laboratory report. For example, an asymptomatic patient with a serum potassium concentration of 3 mEq/L (reference range: 3.5–5 mEq/L) does not cause as much concern as a patient who has a concentration of 3.3 mEq/L but is symptomatic. Tests for occult disease, such as colon cancer, cervical cancer, and hyperlipidemia, are exceptions to this logic because the patients being tested are asymptomatic. Baseline results, rate of change, and patterns should be considered when interpreting laboratory results.

Baseline Results

Baseline studies establish relativity and are especially useful when reference ranges are wide or when reference values vary significantly among patients. For example, lovastatin and other hydroxymethylglutaryl coenzyme A reductase inhibitors cause myopathy and liver dysfunction in a small percentage of patients. The myopathy is symptomatic (muscle pain or weakness) and elevates CK concentrations. The drug-induced liver dysfunction is asymptomatic and causes elevated AST and ALT. Some clinicians establish a pretreatment baseline profile including CK, AST, and ALT and then conduct periodic testing thereafter to identify potential drug-induced toxicity. Creatine kinase has a wide reference range (55–170 units/L);

establishment of a baseline allows the clinician to identify early changes, even within the reference range. The baseline value is also used to establish relative therapeutic goals. For example, the aPTT is used to assess patient response to heparin anticoagulation. Therapeutic targets are expressed in terms of how much higher the patient's aPTT is compared to the baseline control.

Laboratory Value Compared to Reference Range

Not all laboratory values above the ULN require intervention. Risk-to-benefit considerations may require that some evidence of drug-induced organ damage is acceptable given the ultimate benefit of the drug. For example, a six-month course of combination drug therapy including isoniazid, a known hepatotoxin, is recommended for treatment of latent tuberculosis.[50] The potential benefit of at least six months of therapy (i.e., lifetime protection from tuberculosis in the absence of reinfection) means that clinicians are willing to accept some evidence of liver toxicity with continued drug therapy (e.g., isoniazid is continued until AST is greater than five times the ULN in asymptomatic individuals or greater than three times the ULN in symptomatic patients).[51]

Rate of Change

The *rate of change* of a laboratory value provides the clinician with a sense of risks associated with the particular signs and symptoms. For example, a patient whose RBC count falls from 5 million/mm^3 to 3.5 million/mm^3 over several hours is more likely to be symptomatic and need immediate therapeutic intervention than if the decline took place over several months.

Isolated Results Versus Trends

An isolated abnormal test result is difficult to interpret. However, one of several values in a series of results or similar results from the same test performed at two different times suggests a pattern or trend. For example, a random serum glucose concentration of 300 mg/dL (reference range ≤200 mg/dL in adults) might cause concern unless it was known that the patient was admitted to the hospital the previous night for treatment of diabetic ketoacidosis with a random serum glucose of 960 mg/dL. A series of laboratory values adds perspective to an interpretation but may increase overall costs.

Spurious Results

A *spurious laboratory value* is a false laboratory value. The only way to differentiate between an actual and a spurious laboratory value is to interpret the value in context of what else is known about the patient. For example, a serum potassium concentration of 5.5 mEq/L (reference range: 3.5–5 mEq/L) in the absence of significant electrocardiographic changes (i.e., wide, flat P waves, wide QRS complexes, and peaked T waves) and risk factors for hyperkalemia (i.e., renal insufficiency) is most likely a spurious value. Possible causes of falsely elevated potassium, such as hemolysis, acidosis, and laboratory error, have to be ruled out before accepting that the elevated potassium accurately reflects the patient's actual serum potassium. Repeat testing of suspected spurious laboratory values increases the cost of patient care but may be necessary to rule out an actual abnormality.

FUTURE TRENDS

As advances in miniaturization produce smaller and more portable analytical devices, POCT will progress and become more widely available. Real-time, in vivo POCT may become standard in many patient care areas. Laboratory test specificity and sensitivity will improve with more sophisticated testing. Genetic testing (laboratory analysis of human deoxyribonucleic acid [DNA], RNA, chromosomes, and proteins) will undergo rapid growth and development in the next few decades; genetic testing will be able to predict an individual's risk for disease, identify carriers of disease, establish diagnoses, and provide prognostic data. Genetic links for a diverse group of diseases including cystic fibrosis, Down syndrome, Huntington disease, breast cancer, Alzheimer disease, schizophrenia, PKU, and familial hypercholesterolemia are established; genetic links for many additional diseases will be established. Variations in DNA sequences will be well-described and linked to individualized, disease management strategies.[52] Developments in nanotechnology will provide simple and inexpensive in vitro and in vivo assessments. Advances in array-based technologies (i.e., simultaneous evaluation of multiple analytes from one sample) will reduce sample volume and cost.

PATIENT ASSESSMENT

Evaluation of patient laboratory data is an important component of designing, implementing, monitoring, evaluating, and modifying patient-specific medication therapy management plans. Depending on the setting, state laws, and collaborative practice agreements, some pharmacists have the authority to order and assess specific laboratory tests (e.g., drug serum concentrations, SCr, liver function tests, serum electrolytes) or to perform POCT (e.g., lipid screening profiles, prothrombin time, HbA1c, rapid strep test). Pharmacists in ambulatory clinics and acute care inpatient settings have routine access to the same patient laboratory data as all other members of the healthcare team, but many community-based pharmacists do not have access to patient laboratory data. Although lack of access to laboratory data is currently a barrier, the increasing use of electronic patient charts and databases will improve pharmacist access to patient laboratory data.

SUMMARY

Clinical laboratory tests are convenient methods to investigate disease-related and drug-related patient issues, especially because knowledge of pathophysiology and therapeutics alone is insufficient to provide high-quality clinical considerations. This chapter should help clinicians appreciate general causes and mechanisms of abnormal test results. However, results within the reference range are not always associated with an absence of signs and symptoms. Many factors influence the reference range. Knowing the sensitivity, specificity, and predictive value is important in selecting an assay and interpreting its results. Additionally, an understanding of the definitions, concepts, and strategies discussed should also facilitate mastering information in the following chapters.

LEARNING POINTS

1. ***What factors should be considered when assessing a laboratory parameter that is outside the reference range?***

   ANSWER: The upper and lower limits of the reference range are not absolute; by definition, some normal results will fall outside the reference range. Other factors to consider include the sensitivity and specificity of the test, the critical value for the test, the acuity of the change, drug–drug and drug–test interactions, patient signs and symptoms, laboratory error, specimen handling, patient age, and timing of the test.

2. ***What factors should be considered when discussing PSA screening?***

   ANSWER: Sensitivity and specificity should be considered. Although PSA is specific for the prostate, it has a low sensitivity for detecting prostate cancer and is elevated by urethral instrumentation, prostatitis, urinary retention, prostatic needle biopsy, and benign prostatic hyperplasia. Specificity for prostate cancer is lower in older men with benign prostatic hyperplasia than in younger men without prostatic hyperplasia. Thus, an elevated PSA level found during screening may result in unnecessary biopsies, treatment, and complications. Many authorities do not recommend PSA-based screening for prostate cancer.[53]

3. ***What advantages and disadvantages should be considered when recommending at-home laboratory testing kits?***

   ANSWER: Advantages of patient-directed diagnostic and monitoring testing include convenience, cost savings as compared to a physician office visit, quickly available results, and privacy. Disadvantages include lack of information regarding sensitivity, specificity, precision, or accuracy; misinterpretation of the test results; the absence of pretest and posttest counseling; and delays in seeking medical advice. Patients who wish to purchase FDA-approved home-testing kits should be cautioned to seek advice before making treatment decisions based solely on home-testing laboratory results.

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2

INTRODUCTION TO COMMON LABORATORY ASSAYS AND TECHNOLOGY

Nicholas M. Moore

OBJECTIVES

After completing this chapter, the reader should be able to

- Describe the current and developing roles of laboratory testing in accurately diagnosing diseases
- Describe the basic elements of photometry and the major components of a spectrophotometer
- Explain the principles of turbidimetry and nephelometry as applied to laboratory testing
- Describe the analytic techniques of electrochemistry based on potentiometry, coulometry, voltammetry, and conductometry
- Describe the major electrophoresis techniques and their applications
- Describe the major analytic techniques of chromatography and compare gas- and high-performance liquid chromatography with respect to equipment and methodology
- Learn the basic principles of immunoassays; compare and contrast the underlying principles, methods, and tests performed involving radioimmunoassay, enzyme-linked immunosorbent assay, enzyme-multiplied immunoassay, fluorescent polarization immunoassay, and agglutination enzyme-linked immunoassay tests
- Describe the basic components of a mass spectrometry system

(continued on page 20)

THE CHANGING ROLE OF THE LABORATORY IN THE DIAGNOSIS OF DISEASE

Traditionally, the physician bases a clinical diagnosis and patient management protocol on the patient's family and medical history, clinical signs and symptoms, and data derived from laboratory and imaging tests. An accurate history and physical examination of the patient are still considered among the most informative procedures in establishing accurate diagnoses of disease, with clinical laboratory test results playing important roles in confirming and ruling out certain diseases.

Pharmaceutical companies have developed drugs based on these collective empiric observations and known disease mechanisms. Some common examples include medications for high cholesterol, which modify the absorption, metabolism, and generation of cholesterol. Agents have been developed that are aimed at improving insulin release from the pancreas and sensitivity of the muscle and fat tissues to insulin action. Antibiotics are based on the observation that microbes produce substances, which inhibit other species. Hypotensive medications that lower blood pressure have typically been designed to act on physiologic pathways involved in hypertension (such as renal salt and water absorption, vascular contractility, and cardiac output). This has often been a reactive approach with appropriate treatments and therapy starting after the signs and symptoms appear.

The past 30 years has seen remarkable progress in the role of the laboratory in personalizing medicine, a consequence of the advances in human and medical genetics. These advances have enabled a more detailed understanding of the impact of genetics in disease and have led to new disciplines: genomics, epigenetics, and proteomics. It is anticipated that discoveries in these areas will change the practice of traditional clinical medicine into a more personalized medicine approach and will also impact pharmaceutical development.

Many of the traditional laboratory procedures and tests that are described in the following parts of this chapter will create the framework upon which these potential advances will be based—researchers are simplifying them, improving throughput, and running in real time. As these tests are automated, they will take their place alongside current testing procedures. In the United States alone, each year clinicians order laboratory tests costing billions of dollars. Although most laboratory testing is not performed by these clinicians themselves, it is imperative that they have an introduction to, and a basic understanding of, the more common and newer methods and techniques used to generate this clinical data. This understanding is essential for the proper ordering of tests and, most importantly, the correct interpretation of test results. This chapter provides an introduction to these methods and techniques.

Clinical laboratory testing represents a vast array of diverse procedures ranging from microscopic examination of tissue specimens (histopathology), to measurement of cellular components, to the amplification and detection of genetic material, such as the detection of a gene mutation or fusion for malignancies or the identification of antimicrobial resistance genes in bacteria. A consideration of all of the diverse methodologies used in these procedures is beyond the scope of this chapter,

OBJECTIVES

- **Understand the basic principles of the commonly used cytometry systems**
- **Describe the impact of genomics, epigenetics, and proteomics on the personalization of medical practice and the newer roles that laboratory tests will play in the future**
- **Describe the basic principles of molecular diagnostics**
- **Describe the basic techniques of the polymerase chain reaction**

but all share some of the common characteristics of automation and mechanization. The two often intertwine; automation commonly involves the mechanization of basic manual laboratory techniques or procedures such as those described throughout this chapter. The common goals of total laboratory automation (TLA) result in increased efficiency and throughput leading to decreased turnaround times, reduced errors, and the ability to integrate various quality assurance and improvement processes in the laboratory.

AUTOMATION IN THE HOSPITAL AND CLINICAL LABORATORY

This trend toward automation in the hospital and clinical laboratory is, in part, motivated by the drive toward higher productivity and cost efficiency.[1] Another key driver clinical laboratories face is the federal government. According to a report issued from the U.S. Department of Health and Human Services, the Office of the Inspector General (OIG) stated that Medicare could have saved $910 billion (38%) on laboratory test reimbursement if they lowered the reimbursement rate for the top 20 laboratory tests.[2] A final conclusion from this report was the OIG should consider reintroducing competitive bidding and adjusting the reimbursement rate for these laboratory tests. Clinical laboratories, like many other departments in hospitals and other healthcare facilities, are facing the pressure of providing more services, while maintaining high quality standards with a reduced revenue stream. In its most comprehensive sense, TLA encompasses all procedures from receipt of the specimen to the reporting of results. System designs and functionality can vary. They can involve consolidated analyzers, individual or integrated, and automated devices that address specific tasks, coupled to specimen processing and transportation systems, as well as process control software (i.e., middleware) that automates each stage of the system. One plausible vision of the future is that the centralized hospital and clinical laboratory will consist mainly of automated laboratory systems capable of performing high volume and esoteric testing operated by skilled medical laboratory scientists.[3,4]

Laboratory automation involves a variety of steps and generally begins with processes that are manual in nature: obtaining the specimen, patient identification, transportation, and any preanalytic specimen processing. Once in the laboratory a quality control (QC) process begins with a check of the preordered specimen to ensure that specimens have correct identification labels and bar codes, the correct tube was used for the blood test ordered, and appropriate quality and adequate quantity of material is provided for the testing requested. The TLA systems are currently capable of performing only some of the above preanalytic checks. Determining whether, for example, a specimen is grossly hemolyzed, icteric, or lipemic usually requires examination by a laboratory scientist.

In many divisions of the centralized laboratory, three major areas (e.g., chemistry analyzers, hematology analyzers, and automated microbial identification systems) generate information in almost completely automated ways. Using the example of a chemistry analyzer, introduction of a specimen begins with aspiration of the sample into a continuous-flow system. Each specimen passes through the same continuous stream and is subjected to the same various analytical reactions. In some systems, the use of repeated flushing and washing steps of probes within the systems prevents carryover between specimens, while other systems use discrete specimen sampling through the use of disposable pipet tips. Many results generated by automated chemistry analyzers rely on reactions based on principles of photometry, which will be discussed later in this chapter. In addition to the more commonly requested serum chemistry levels, enzymes, therapeutic drugs, hormones, and other analytes can also be measured using these techniques.

All modern automated analyzers rely on computers and sophisticated software to perform these sample processing functions. Calculations (statistics on patient or control values), monitoring (linearity and QC), and display (acquisition and collation of patient results and warning messages and delta checks) functions are routinely performed by these instruments once the specimen has been processed. Automation does not end at this stage. Many centralized laboratories have electronic interfaces that link separate analyzers to the laboratory information system (LIS). In turn, the LIS is interfaced with the hospital electronic medical record system. This interface allows for vital two-way connectivity between the two systems. Laboratory orders are automatically sent to the LIS. This prevents errors when a manual requisition system is utilized. Also, laboratory diagnostic information can be immediately uploaded into the patient chart for review by the clinician once results are verified manually by a medical laboratory scientist or through the use of automated rule systems developed by the laboratory. Then, based on the results generated, some laboratories have created electronic rules that can automatically order repeat and reflex testing, track samples and results through the system, and manage the storage and, when necessary, the retrieval of specimens.

Standardization within the laboratory automation arena is an essential means of assuring QC and quality assurance for the diagnostic data. The Clinical and Laboratory Standards Institute is an organization that uses a consensus-based approach in developing a series of comprehensive standards and guidelines that serves as the "gold standard" for laboratory automation.[5]

The discipline of informatics is a parallel component of laboratory automation. As generators and collectors of information, large laboratories provide relevant clinical information to a wide network of physicians and other healthcare professionals in an efficient manner. Informatics in the laboratory involves the use of collected data for the purposes of healthcare decision making. Modern LIS have the capability of analyzing data in a variety of ways that enhance patient care. The ability to transmit and share such information over the Internet is becoming as indispensable a function of the laboratory as performing the tests themselves. Some laboratories and healthcare systems have implemented patient access portals where patients can have limited access to their healthcare information, including laboratory test results after physicians have reviewed these results. The portals will become centers of information management for hospital-based medicine practice as well as for the community. In parallel with the development of the highly automated core laboratory, technological advances in miniaturization of analyzers will make point-of-care testing an essential and complementary tool for the diagnosis and treatment of disease.

PHOTOMETRY

Photometry is the measurement of light. Light is how we define the visible radiant energy from the ultraviolet (UV) and visible portions of the electromagnetic spectrum. The wavelength of light is often expressed in nanometers (nm). Humans can only naturally perceive a very limited range of about 380–750 nm (**Table 2-1**). Modern clinical laboratory instruments, however, can accurately measure the absorbance or emittance between 150 (the low UV) and 2500 nm (the near infrared region).[9] These instruments are classified by the source of light as well as whether the light is absorbed or emitted. Four types of photometric instruments are currently in use in laboratories: molecular absorption, molecular emission (fluorometers), atomic emission (flame photometers), and atomic absorption spectrophotometers.

TABLE 2-1. Wavelength Characteristics of Ultraviolet, Visible, and Infrared Light

WAVELENGTH (nm)	COLOR OBSERVED	REGION
<380	Invisible	Ultraviolet
390–440	Violet	Visible
440–500	Blue	Visible
500–580	Green	Visible
580–600	Yellow	Visible
600–620	Orange	Visible
620–750	Red	Visible
>800	Not visible	Infrared

Molecular Absorption Spectrophotometers

Molecular absorption spectrophotometers, usually referred to as *spectrophotometers*, are commonly employed in conjunction with other methodologies, such as nephelometry, which is discussed below, and enzyme immunoassay (EIA). In spectrophotometry, analyzers measure the intensity of light at selected wavelengths. Spectrophotometers are easy to use, have relatively high specificity, produce highly accurate results, and are capable of generating both qualitative and quantitative data. The high specificity and accuracy are obtained by isolated analytes reacting with various substances that produce colorimetric reactions.

The basic components of two types of spectrophotometers (single and double beam) are depicted in **Figure 2-1**. Single-beam instruments have a light source (I) (e.g., a tungsten bulb or laser), which passes through an entrance slit that minimizes stray light. Specific wavelengths of light are selected by the use of a monochromator (II). Light of a specific wavelength then passes through the exit slit and illuminates the contents of the analytical cell or cuvette (III). After passing through the test solution, the light strikes a detector, usually a photomultiplier tube (IV). This tube amplifies the electronic signal, which is then sent to a recording device (V). The result is then compared with a standard curve to yield a specific concentration of analyte.

The double-beam instrument, similar in design to single-beam instruments, is designed to compensate for changes in absorbance of the reagent blank and light source intensity. It utilizes a mirror (VI) to split the light from a single source

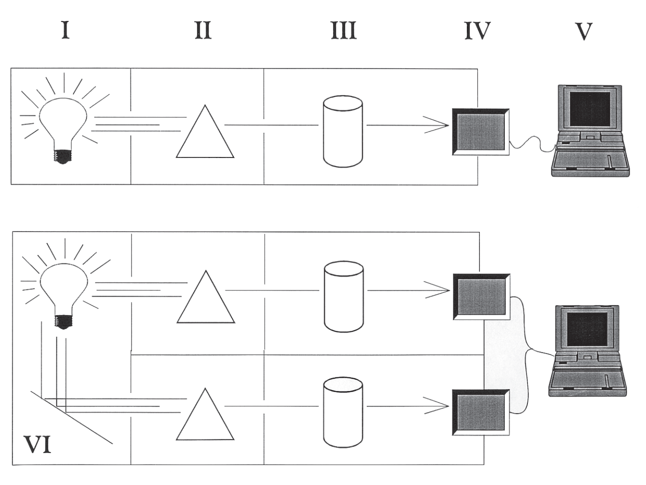


FIGURE 2-1. Schematic of single-beam (upper portion) and double-beam (lower portion) spectrophotometers. I = radiant light source; II = monochromator; III = analytical cuvette; IV = photomultiplier; V = recording device; VI = mirror.

into two beams, one passing through the test solution and one through the reagent blank. By doing so, it automatically corrects optical errors as the wavelength changes.

Most measurements are made in the visible range of the spectrum, although sometimes measurements in the UV and infrared ranges are employed. The greatest sensitivity is achieved by selecting the wavelength of light in the range of maximum absorption. If substances are known to interfere at this wavelength, measurements may be made at a different wavelength in the absorption spectrum. This modified procedure allows detection or measurement of the analyte with minimal interference from other substances.

Molecular Emission Spectrophotometers

Molecular emission spectrophotometry is usually referred to as *fluorometry*. The technology found in these instruments is based on the principle of luminescence: an energy exchange process that occurs when electrons absorb electromagnetic radiation and then emit this excited energy level at a lower level (e.g., longer wavelength). An atom or molecule that fluoresces is termed a *fluorophore*. Three types of photoluminescence techniques—fluorescence, phosphorescence, and chemiluminescence—form the principle on which these sensitive clinical laboratory instruments operate.

Fluorescence results from a three-stage process that occurs in fluorophores. The first stage involves the absorption of radiant energy by an electron in the ground state creating an excited singlet state. During the very short lifetime of this state (order of nanoseconds), energy from the electronic-vibrational excited state is partially dissipated through a radiationless transfer of energy that results from interactions with the molecular environment and leads to the formation of a relaxed excited singlet state. This is followed by relaxation to the electronic ground state by the emission of radiation (fluorescence). Because energy is dissipated, the energy of the emitted photon is lower and the wavelength is longer than the absorption photon. The difference between these two energies is known as *Stokes shift*. This principle is the basis for the sensitivity of the different fluorescence techniques because the emission photons can be detected at a different wavelength than the excitation photons. Consequently, the background is lower than with absorption spectrophotometry where the transmitted light is detected against a background of incident light at the same wavelength.[9]

The phenomenon of *phosphorescence* is similar to fluorescence because it also results from the absorption of radiant energy by a molecule; however, it is also a competitive process. Unlike fluorescence, which results from a singlet-singlet transition, phosphorescence is the result of a triplet-singlet transition. When a pair of electrons occupies a molecular orbital in the ground or excited state, a singlet state is created. When the electrons are no longer paired, three different arrangements are possible, each with a different magnetic moment, the triplet state. The electronic energy of a triplet state is lower than a singlet state; therefore, when the relaxed excited singlet state overlaps with a higher triplet state, energy may be transferred through a process called *intersystem crossing*. As in the case of an excited singlet state, energy may be dissipated through several radiationless mechanisms to the electronic ground state; however, when a triplet-singlet transition occurs, the result is phosphorescence. The probability of this type of transition is much lower than a singlet-singlet transition (fluorescence), and the emission wavelength and decay times are also longer than for fluorescence emission. Because the various forms of radiationless energy transfer compete so effectively, phosphorescence is generally limited to certain molecules, such as many aromatic and organometallic compounds, at very low temperatures, or in highly viscous solutions.[10,11]

The phenomenon of *chemiluminescence* is also similar to that of fluorescence in that it results from light emitted from an excited singlet state. However, unlike both fluorescence and phosphorescence, the excitation energy is caused by a chemical or electrochemical reaction. The energy is typically derived from the oxidation of an organic compound, such as luminol, luciferin, or an acridinium ester. Light is derived from the excited products that are formed in the reaction.

Different instruments have been developed that use these basic principles of luminescence. These devices use similar basic components along the following pathway: a light source (laser or mercury arc lamp), an excitation monochromator, a sample cuvette, an emission monochromator, and a photodetector.[9] Although the principles of these instruments are relatively straightforward, various modifications have been developed for specific applications.

An important example is fluorescent polarization in fluorometers. Fluorescent molecules (fluorophores) become excited by polarized light when the plane of polarization is parallel to their absorption transition vector, provided the molecule remains relatively stationary throughout the excited state. If the molecules rotate rapidly, light will be emitted in a different plane than the excitation plane. The intensity of light emitted by the molecules in the excitation polarization plane and at 90° permits the fluorescence polarization to be measured. The degree to which the emission intensity varies between the two planes of polarization is a function of the mobility of the fluorophore. Large molecules move slowly during the excited state and will remain highly polarized. Small molecules that rotate faster will emit light that is depolarized relative to the excitation plane.[11]

One of the most common applications of fluorescence polarization is competitive immunoassays, used to measure a wide range of analytes including therapeutic and illicit drugs, hormones, antigens and antibodies. This important methodology involves the addition of a known quantity of fluorescent-labeled analyte molecules to a serum antibody (specific to the analyte) mixture. The labeled analyte will emit depolarized light because its motion is not constrained. However, when it binds to an antibody, its motion will decrease and the emitted light will be more polarized. When an unknown quantity of an unlabeled analyte is added to the mixture, competitive binding for the antibody will occur and reduce the polarization of the labeled analyte. By using standard curves of known drug concentrations versus polarization, the concentration of the unlabeled analyte can be determined.[11]

Atomic Emission and Atomic Absorption Spectrophotometers

Atomic absorption (AA) spectrophotometry has limited use in most modern clinical laboratories, and AA spectrophotometry procedures are currently associated mainly with toxicology laboratories where poisonous substances, such as lead and arsenic, need to be identified. In this technique, the element is dissociated from its chemical bonds (atomized) where it is placed into an unexcited ground state (neutral atom). In this state, the element is in its lowest energy state and capable of absorbing energy in a narrow range that corresponds to its line spectrum.[12] Generally speaking, AA spectrophotometry methods have greater sensitivity compared to flame emission methods. Furthermore, due to the specificity of the wavelength from the cathode lamp, AA methods are much more specific for the element being measured.[33]

TURBIDIMETRY AND NEPHELOMETRY

When light passes through a solution, it can be either absorbed or scattered. The basis for measuring light-scatter has been applied to various immunoassays for specific proteins or haptens. *Turbidimetry* is the technique for measuring the percent light absorbed. In this method, the turbidity of a solution decreases the intensity of the incident light beam as it passes through particles in solution. A major advantage of turbidimetry is that measurements can be made with laboratory instruments (e.g., a spectrophotometer) used for other procedures in laboratory testing. Errors associated with this method usually involve sample and reagent preparation. For example, because the amount of light blocked depends on both the concentration and size of each particle, differences in particle size between the sample and the standard is one cause of error. The length of time between sample preparation and measurement, another cause of error, should be consistent because particles settle to varying degrees, allowing more or less light to pass. Large concentrations are necessary because this test measures small differences in large numbers.

Nephelometry, which is similar to turbidimetry, is a technique that is used for measuring the scatter of light by particles. The main differences are that (1) the light source is usually a laser, and (2) the detector, used to measure scattered light, is at a right angle to the incident light. Beam light scattered by particles is a function of the size and number of the particles. Nephelometric measurements are more precise than turbidimetric ones as the smaller signal generated for low analyte concentrations is more easily detected against a very low background.[13] Because antigen–antibody complexes are easily detected by this method, it is commonly employed in combination with EIAs.

REFRACTOMETRY

Refractometry measurements are based on the principle that light bends as it passes through different media. The ability of a liquid to bend light depends on several factors: wavelength of the incident light, temperature, physical characteristics of the medium, and the solute concentration in the medium. By keeping the first three parameters constant, refractometers can measure the total solute concentration of a liquid. This procedure is particularly useful, especially as a rapid screening test because no chemical reagents and reactions are involved.[9]

Refractometers are commonly used to measure total dissolved plasma solids (mostly proteins) and urine specific gravity. In the refractometer, light is passed through the sample and then through a series of prisms. The refracted light is projected on an eyepiece scale. The scale is calibrated in grams per deciliter for serum protein, and in the case of urine, for specific gravity. In the eyepiece, a sharp line of demarcation is apparent and represents the boundary between the sample and distilled water. In the case of plasma samples, the refraction angle is proportional to the total dissolved solids. Although proteins are the predominant chemical species, other substances such as electrolytes, glucose, lipids, and urea contribute to the refraction angle. Therefore, measurements made on plasma do not correlate exactly to the true protein concentrations, but as the nonprotein solutes contribute to the total solutes in a predictable manner, accurate corrections are possible.[14]

OSMOMETRY

In the clinical laboratory, *osmometer* readings are interpreted as a measure of total concentration of solute particles and are used to measure the osmolality of biological fluids such as serum, plasma, or urine. When osmotically active particles are dissolved in a solvent (water, in the case of biological fluids), four physical properties of the water are affected: the osmotic pressure and the boiling point are increased, and the vapor pressure and the freezing point are decreased. Because each property is related, they can be expressed mathematically in terms of the others (colligative properties) and to osmolality. Consequently, several methods can be used to measure osmolality including freezing-point depression, colloid osmotic pressure (COP), and vapor pressure osmometry.[15]

The most commonly used devices to measure osmolality or other colligative properties of a solution are freezing-point depression osmometers. In these analyzers, the sample is rapidly cooled several degrees below its freezing point in the cooling chamber. The sample is stirred to initiate freezing of the super-cooled solution. When the freezing point of the solution is reached (the point where the rate of the heat of fusion released by ice formation comes into equilibrium with the rate of heat removal by the cooling chamber), the osmolality can be calculated.[9]

In certain situations, it is important to measure the COP, a direct measure of the contribution of plasma proteins to the osmolality. Because of the large molecular weight of plasma proteins, their contribution to the total osmolality is very small as measured by freezing-point depression and vapor pressure osmometers. As a low COP favors a shift of fluid from the intravascular compartment to the interstitial compartment, measurement of the COP is particularly important in monitoring intravascular volume and useful in guiding fluid therapy in different circumstances to prevent peripheral and pulmonary edema.

The *COP osmometer*, also known as a *membrane osmometer*, consists of two fluid-filled chambers separated by a semipermeable membrane. One chamber is filled with a colloid-free physiologic saline solution that is in contact with a pressure transducer. When the plasma or serum is placed in the sample chamber, fluid moves by osmosis from the saline chamber to the sample chamber, thus causing a negative pressure to develop in the saline chamber. The resultant pressure is the COP.[15]

ELECTROCHEMISTRY

In the clinical laboratory, analytic electrochemical techniques involve the measurement of current or voltage produced by the activity of different types of ions. These analytic techniques are based on the fundamental electrochemical phenomena of potentiometry, coulometry, voltammetry, and conductometry.

Potentiometry

Potentiometry involves the measurement of electrical potential differences between two electrodes in an electrochemical cell at zero current flow. This method is widely used in both laboratory-based analyzers and point-of-care analyzers to measure pH, pCO_2, and electrolytes in whole blood samples. This electrochemical method is based on the Nernst equation, which relates the potential to the concentration of an ion in solution, to measure analyte concentrations.[16] Each electrode or half-cell in an electrochemical cell consists of a metal conductor that is in contact with an electrolyte solution. One of the electrodes is a reference electrode with a constant electric potential; the other is the measuring or indicator electrode. The boundaries between the ion conductive phases in the cell determine the type of potential gradients that exist between the electrodes and are defined as redox (oxidation reduction), membrane, and diffusion potentials.

A redox potential occurs when the two electrolyte solutions in the electrochemical cell are brought into contact with each other by a salt bridge so that the two solutions can achieve equilibrium. A potentiometer may be used to measure the potential difference between the two electrodes. This is known as the *redox potential difference* because the reaction involves the transfer of electrons between substances that accept electrons (oxidant) and substances that donate electrons (reductant). Junctional potentials rather than redox potentials occur when either a solid state or liquid interface exists between the ion conductive phases. These produce membrane or diffusion potentials, respectively. In each case the concentration of an ion in solution can be measured using the Nernst equation, which relates the electrode potential to the activity of the measured ions in the test solution[9]: $E = E^0 – (0.059/z)\log (C_{red}/C_{ox})$ where E = the total potential (in mV), E^0 = is the standard reduction potential, z = the number of electrons involved in the reduction reaction, C_{red} = the molar concentration of the ion in the oxidized form, and C_{ox} = the molar concentration of the ion in the reduced form.

Ion-selective electrodes (ISEs) consisting of a membrane that separates the reference and test electrolyte solutions are very selective and sensitive for the ions that they measure. For this reason further discussion on potentiometry will focus on these types of electrodes. The ISE method, having comparable or better sensitivity than flame photometry, has become the principal test for determining urine and serum electrolytes in the clinical laboratory. Typically, ion concentrations such as sodium, potassium, chloride, calcium, and lithium, are measured using this method (**Table 2-2**).

The principle of ISE involves the generation of a small electrical current when a particular ion comes in contact with an electrode. The electrode selectively binds the ion to be measured. To measure the concentration, the circuit must be completed with a reference electrode. The three types of electrodes are ion-selective glass membranes, solid-state electrodes, and liquid ion-exchange membranes. As shown in **Figure 2-2**, ion-selective glass membranes preferentially allow hydrogen (H^+), sodium (Na^+), and ammonium (NH_4^+) ions to cross a hydrated outer layer of glass. The H^+ glass electrode or pH electrode is the most common electrode for measuring H^+. Electrodes for Na^+, potassium (K^+), lithium (Li^+), and NH_4^+ are also available. An electrical potential is created when these ions diffuse across the membrane.

Solid-state electrodes consist of halide-containing crystals for measuring specific ions. An example is the silver–silver chloride electrode for measuring chloride.[9] Liquid ion-exchange membranes contain a water-insoluble, inert solvent that can dissolve an ion-selective carrier. Ions outside the membrane produce a concentration-related potential with the ions bound to the carrier inside the membrane.[9] The electrodes are separated from the sample by a liquid junction or salt bridge. Because the liquid junction generates its own voltage at the sample interface, it is a source of error. This error is overcome by adjusting the composition of the liquid junction.[17] Overall, this method is simple to use and more accurate than flame photometry for samples having low plasma water due to conditions such as hyperlipoproteinemia.[18]

Compared to other techniques, such as flame photometry, ISEs are relatively inexpensive and simple to use and have an extremely wide range of applications and wide concentration range. They are also very useful in biomedical applications because they measure the activity of the ion directly in addition to the concentration.

TABLE 2-2. Common Laboratory Tests Performed with Various Assays

ASSAY	ANALYSIS TIME (MIN)	COMMON TESTS	USE
ISE	6–18	Electrolytes, (sodium potassium, chloride, calcium, lithium, total carbon dioxide)	Primary testing method
GC	30	Toxicologic screens, organic acids, drugs (e.g., benzodiazepines and TCAs)	Primary testing method
HPLC	30	Toxicologic screens, vanilmandelic acid, hydroxy-vanilmandelic acid, amino acids, drugs (e.g., indomethacin, anabolic steroids, cyclosporin)	Primary and secondary or confirmatory testing methods
ELISA	0.1–0.3	Serologic tests (e.g., ANA; rheumatoid factor; hepatitis B, cytomegalovirus, and human immunodeficiency virus antigens/antibodies)	Primary testing method
EMIT	0.1–0.3	Therapeutic drug monitoring, (e.g., aminoglycosides, vancomycin, digoxin, antiepileptics, antiarrhythmics, theophylline), toxicology/drugs of abuse testing (acetaminophen, salicylate, barbiturates, TCAs, amphetamines, cocaine, opiates)	Primary testing method
FPIA	0.1–0.2	Therapeutic drug monitoring (e.g., aminoglycosides, vancomycin, antiepileptics, antiarrhythmics, theophylline, methotrexate, digoxin, cyclosporine), thyroxine, triiodothyronine, cortisol, amylase, cholesterol, homocysteine	Primary testing method
PCR	0.1–0.2	Microbiologic and virologic markers of organisms and genetic markers	Primary testing method

ANA = antinuclear antibody; ELISA = enzyme-linked immunosorbent assay; EMIT = enzyme-multiplied immunoassay technique; FPIA = fluorescent polarization immunoassay; GC = gas chromatography; HPLC = high-performance liquid chromatography; ISE = ion-selective electrode; PCR = polymerase chain reaction; TCAs = tricyclic antidepressants.

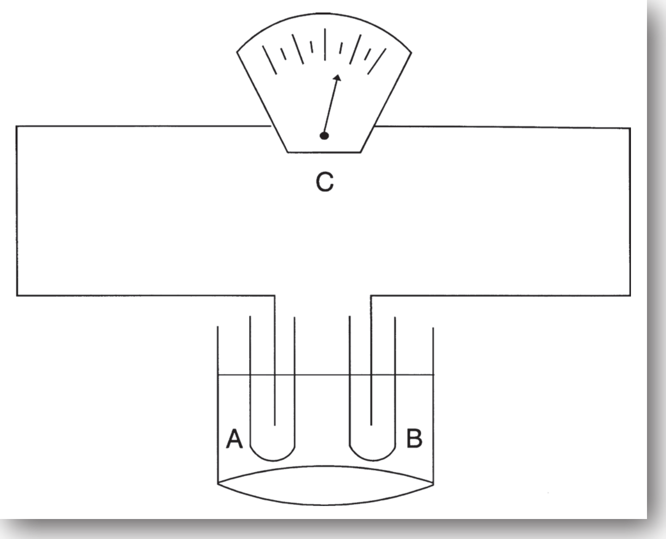


FIGURE 2-2. The pH meter is an example of a test that uses ISE to measure the concentration of hydrogen ions. An electric current is generated when hydrogen ions come in contact with the ISE (A). The circuit is completed through the use of a reference electrode (B) submerged in the same liquid as the ISE (also known as the *liquid junction*). The concentration can then be read on a potentiometer (C).

Coulometry

Coulometry is an analytical method for measuring an unknown concentration of an analyte in solution by completely converting the analyte from one oxidation state to another. This is accomplished through a form of titration where a standardized concentration of the titrant is reacted with the unknown analyte, requiring no chemical standards or calibration. The point at which all of the analyte has been converted to the new oxidation state is called the *endpoint* and is determined by some type of indicator that is also present in the solution.

This technique is based on the Faraday law, which relates the quantity of electric charge generated by an amount of substance produced or consumed in the redox process and is expressed as $znF = It = Q$ where z is the number of electrons involved in the reaction, n is the quantity of the analyte, F is the Faraday constant (96,487 C/mol), I is the current, t is time, and Q is the amount of charge that passes through the cell.

Coulometry is often used in clinical applications to determine the concentration of chloride in clinical samples. The chloridometer is used to measure the chloride ion (Cl^-) concentration in sweat, urine, and CSF samples.[9] The device uses a constant current across two silver electrodes. The silver ions (Ag^+) that are generated at a constant rate react with the Cl^- ions in the sample. The reaction that produces insoluble AgCl ceases once excess Ag^+ ions are detected by an indicator and reference electrodes. Because the quantity of Ag^+ ions generated is known, the quantity of Cl^- ions may be calculated using the Faraday law.

Voltammetry

Voltammetry encompasses a group of electrochemical techniques in which a potential is applied to an electrochemical cell with the simultaneous measurement of the resulting current. By varying the potential of an electrode, it is possible to oxidize and reduce analytes in a solution. At more positive potentials, the electrons within the electrode become lower in energy and the oxidation of species in a solution becomes more likely. At lower potentials, the opposite occurs. By monitoring the current of an electrochemical cell at varying electrode potentials, it is possible to determine several parameters such as concentration, reaction kinetics, and thermodynamics of the analytes.[15]

This technique differs from potentiometry in a number of important ways. Voltammetric techniques use an externally applied force (potential) to generate a signal (current) in a way that would not normally occur, whereas in potentiometric techniques the analytical signal is produced internally through a redox reaction. The electrode arrangement is also quite different between the two techniques. To analyze both the potential and the resulting current, three electrodes are employed in voltammetric devices. The three electrodes include the working, auxiliary, and reference electrodes, which (when connected through a voltmeter) permit the application of specific potential functions. The measurement of the resulting current can yield results about ionic concentrations, conductivity, and diffusion. The ability to apply different types of potential functions or waveforms has led to the development of different voltammetric techniques: linear potential sweep polarography, pulse polarography, cyclic voltammetry, and anode stripping voltammetry.[9] These analytical methods, though not commonly used in clinical laboratories, are very sensitive (detection limits as low as the parts per billion range) and are capable of identifying trace elements in patient tissues such as hair and skin.

Conductometry

Conductometry is the measurement of current flow (proportional to conductivity) between two nonpolarized electrodes of which a known potential has been established. Clinical applications include urea estimation through the measurement of the rate-of-change of conductance that occurs with the urease-catalyzed formation of NH_4^+ and bicarbonate (HCO_3^-). The technique is limited at low concentrations because of the high conductance of biological fluids. Perhaps the most important application of impedance (inversely proportional to conductance) measurements in the clinical laboratory involves the Coulter principle for the electronic counting of blood cells. This method is discussed in detail in the cytometry section.

ELECTROPHORESIS

Electrophoresis is a common laboratory technique, with applications in various clinical laboratory disciplines. Routine diagnostic applications of electrophoresis technology exist for infectious diseases, malignancies, genetic diseases, paternity testing, forensic analysis, and tissue typing for transplantation. Electrophoresis involves the separation (i.e., migration) of charged solutes or particles based on its size. Briefly, samples are applied to an electric field in a solution or in a support medium (i.e., agarose gel) and exposed to a current electric field for a set duration of time. The migration of molecules within the support medium when exposed to the electrical field is dependent on the overall molecular charge, shape, and size of the molecule being studied.[19] Because most molecules of biologic importance are both water-soluble and charged, this analytical tool is one of the most important techniques for molecular separation in the clinical laboratory. The main types of electrophoresis techniques used in both clinical and research laboratories include cellulose acetate, agarose gel, polyacrylamide gel, isoelectric focusing (IEF), two-dimensional (2-D), and capillary electrophoresis (CE). Because of a large number of clinical applications, electrophoresis apparatus, cellulose acetate and agarose gels, and reagents are available from commercial suppliers for each of these specific applications.

The primary application of electrophoresis is the analysis and purification of very large molecules such as proteins and nucleic acids. Electrophoresis also can be applied to the separation of smaller molecules, including charged sugars, amino acids, peptides, nucleotides, and simple ions. Through the proper selection of the medium for electrophoretic separations, extremely high resolution and sensitivity of separation can be achieved. Electrophoretic systems are usually combined with highly sensitive detection methods to monitor and analyze the separations that suit the specific application.[20]

The basic electrophoresis apparatus consists of a high voltage direct power supply that provides the electrical current, electrodes, a buffer, and a support for the buffer or a capillary tube. The support medium used provides a matrix that facilitates separation of the particles. Common support matrices include filter paper, cellulose acetate membranes, agarose, and polyacrylamide gels. When an electrostatic force (EOF) is applied across the electrophoresis apparatus, the charged molecules will migrate to the anode or the cathode of the system depending on their charge. The force that acts on these molecules is proportional to the net charge on the molecular species and the applied voltage (electromotive force). This relationship is expressed as $F = qE/d$ where F is the force exerted on the charged molecule, q is its net charge, E is the electromotive force, and d is the distance across the electrophoretic medium.[15]

Although the basic principles are simple, procedures employed in the electrophoresis process are considerably more complex. Molecules to be separated must be dissolved in a buffer that contains electrolytes, which carry the applied current and fix the pH. The mobility of the molecules will be affected locally by the charge of the electrolytes, the viscosity of the medium, their size, and degree of asymmetry. These factors are related by the following equation: $\mu = q/6\eta r$ where μ is the electrophoretic mobility of the charged molecule, q is its net charge, η is the viscosity of the medium, and r is the ionic radius.[21]

The conditions in which this process occurs are further complicated by the use of a support medium, necessary to minimize diffusion and convective mixing of the bands (caused by the heated current flowing through the buffer). Media used include polysaccharides (cellulose and agarose) and synthetic media such as polyacrylamide. The porosity of these media will, to a large extent, determine the resistance to movement for different ionic species. Therefore, the type of support medium used depends on the application. The above cited factors affecting the process of electrophoresis are controllable and provide optimal resolution for each specific application.

Gel Electrophoresis

Cellulose Acetate and Agarose Gel Electrophoresis

Cellulose acetate and *agarose gel electrophoresis* are methods commonly used in many clinical laboratories for both serum protein and hemoglobin separations. Serum protein electrophoresis is often used as a screening procedure for the detection of disease states, such as inflammation, protein loss, monoclonal gammopathies, and other dysproteinemias. When the molecules have been separated into bands, specific stains can then be used to visualize them. Densitometry is typically used to quantify each band. When a monoclonal immunoglobulin (Ig) pattern is identified, another technique, immunofixation electrophoresis, is used to quantify IgG, IgA, IgM, IgD, and IgE that are present in the specimen. Once these proteins are separated on an agarose gel, specific antibodies directed at the immunoglobulins. The sample is then fixed and stained to visualize and quantify the bands.[22] Separation of proteins may also be accomplished with IEF where the proteins migrate through a stable pH gradient with the pH varying in the direction of migration. Each protein moves to its isoelectric point (i.e., the point where the protein's charge becomes zero and migration ceases). This technique is often used for separation of isoenzymes and hemoglobin variants.

Hemoglobin electrophoresis is the most common method for the screening for the presence of abnormal hemoglobin protein variants (hemoglobinopathies), of which more than 1000 have been described. These variant forms of hemoglobin are often the result of missense mutations in the various globin genes (α, β, γ, or δ) due to a single nucleotide substitution. These mutations can have different manifestations, including altering the structure, stability, synthesis or function of the globin protein. The vast majority of these mutations do not manifest with clinical or hematological problems but some do, such as hemoglobin S (sickle cell disease).

Normal adult hemoglobin is composed of two α-subunits and two β-subunits ($\alpha 2\beta 2$), and comprises approximately 97% of the total hemoglobin. Hemoglobin proteins are separated on a cellulose acetate membrane at an alkaline pH (8.6) initially and then on an agarose gel at an acid pH (6.2). Electrophoresis at both pH conditions is performed for optimal resolution of comigrating hemoglobin bands that occur at either of the pH conditions. For example, hemoglobin S, which causes patients to have sickle cell disease or sickle cell trait, comigrates with hemoglobins D and G at pH 8.6, but it can be separated at pH 6.2. The choice of support media is determined by the resolution of the hemoglobin bands that are achieved. Following electrophoresis, the bands are stained for visualization and the relative proportions of the hemoglobins are obtained by densitometry.[23]

Electrophoresis is also an important technique used in the laboratory where it is used to separate deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and protein fragments. Three common techniques used are Southern, Northern, and Western blots. These techniques differ in the target molecules that are separated. Southern blots separate DNA that is cut with restriction endonucleases and then identified with a labeled (usually radioactive) DNA probe. Northern blots separate fragments of RNA that are probed with labeled DNA or RNA. Western blots separate proteins that are probed with radioactive or enzymatically-tagged antibodies.

Each method involves a series of steps that leads to the detection of the various targets. Following electrophoresis, typically performed with an agarose or polyacrylamide gel, the molecules are transferred to a solid stationary support during the probe hybridization, washing, and detection stages of the assay. The DNA, RNA, or protein in the gel may be transferred onto nitrocellulose paper through electrophoresis or capillary blotting. In the former method the molecules, by virtue of their negative charge, are transferred by electrophoresis. The latter method involves layering the gel on wet filter paper with the nitrocellulose paper on top. Dry filter paper is placed on the nitrocellulose paper and the molecules are transferred with the flow of buffer from the wet to dry filter paper via capillary action. Following the transfer, the nitrocellulose paper is soaked in a blocking solution containing high concentrations of DNA, RNA, or protein. This prevents the probe from randomly sticking to the paper during hybridization. During the hybridization stage, the labeled DNA, RNA, or antibody is incubated with the blot where binding with the molecular target occurs. The probe-target hybrids are detected following a wash step to remove any unbound probe.

Two-Dimensional Electrophoresis

Two-dimensional electrophoresis (2-D electrophoresis) is a powerful and widely used method in proteomics for the analysis of complex protein mixtures extracted from cells, tissues, or other biological samples. Proteins are sorted according to two independent properties: IEF, which separates proteins according to their isoelectric points, and SDS-polyacrylamide gel electrophoresis, which separates proteins according to their molecular weights. Each spot on the resulting two-dimensional array corresponds to a single protein species in the sample.[24] Using this technique, thousands of different proteins can be separated through the use of Coomassie dyes, silver staining, radiography, or fluorographic analysis; quantified; and characterized. Additionally, this technology can be used to explore protein families and search for differences, either genetic or disease-based.

Capillary Electrophoresis

Capillary electrophoresis (CE) includes diversified analytical techniques, such as capillary zone electrophoresis (CZE), capillary gel electrophoresis (CGE), capillary chromatography, capillary IEF, micelle electrokinetic capillary chromatography, and capillary isotachophoresis. Currently, only the first two in the above list have practical applications in the clinical laboratory. Although historically a research tool, CE is being adapted for various applications in the clinical laboratory because of its rapid and high-efficiency separation power, diverse applications, and potential for automation. The possibility of CE becoming an important technology in the clinical laboratory is illustrated by its use in the separation and quantification of a wide spectrum of biological components ranging from macromolecules (proteins, lipoproteins, and nucleic acids) to small analytes (amino acids, organic acids, or drugs).

The CE apparatus consists of a small-bore, silica-fused capillary (25–75 µm), approximately 50–100 cm in length, connected to a detector at one end, and via buffer reservoirs to a high-voltage power supply (25–35 kV) at the other end.[25] Because the small capillaries efficiently dissipate the heat, high voltages can be used to generate intense electric fields across the capillary to produce efficient separations with short separation times. In a CE separation, a very small amount of the sample (0.1–10 nL) is required. When the sample solution is injected into the apparatus, the molecules in the solution migrate through the capillary due to its charge in an electric field (electrophoretic mobility) or due to EOF. The negatively charged surface of the silica capillary attracts positive-charged ions in the buffer solution, which in turn migrate toward the cathode and carry solvent molecules in the same direction. The overall movement of the solvent is called *electroosmotic flow*. The separated proteins are eluted from the cathode end of the capillary. Quantitative detectors such as fluorescence, absorbance, electrochemical detectors, and mass spectrometry (MS) can be used to identify and quantify the proteins in the solution in amounts as little as 10–20 mol of substance in the injected volume.[25] Two major advantages of using CE platforms include the ability to apply higher voltages than traditional electrophoresis platforms and its ease of automation.

Capillary Zone Electrophoresis

Capillary zone electrophoresis (CZE) is the most widely-used type of CE and is used for the separation of both anionic and cationic solutes, usually in a single analysis. In CZE, the anions and cations migrate in different directions, but they both rapidly move toward the cathode due to EOF, which is usually significantly higher than the solute velocity. Therefore, all molecules, regardless of their charge, will migrate to the cathode. In this way the negative, neutral, and positive species can be detected and separated. Common clinical applications include high-throughput separation of serum and urine protein and hemoglobin variants. In the future, other applications will become more commonplace. However, these systems are expensive, and currently conventional methods are used.

Capillary Gel Electrophoresis

Capillary gel electrophoresis (CGE) is the CE analog of traditional gel electrophoresis and is used for the size-based separation of biological macromolecules such as oligonucleotides, DNA restriction fragments, and proteins. The separation is performed by filling the capillary with a sieve-like matrix such as polyacrylamide or agarose to reduce the EOF. Therefore, larger molecules such as DNA will move more slowly resulting in better separation. Although CGE electrophoresis is primarily used in research, clinical applications are beginning to be developed.

Pulsed-Field Gel Electrophoresis

In *pulsed-field gel electrophoresis* (PFGE), power is alternately applied to different pairs of electrodes so that the electric field is cycled through different directions. As the field adjusts direction, molecules reorient themselves to the new field before migration continues through the agarose gel. This technique has been widely used to permit the separation of very large molecules, such as DNA fragments larger than 50 kb. Also, PFGE has been used for typing various strains of bacterial DNA after the genetic material has been cut with a particular restriction enzyme (the cut DNA provides a unique bacterial fingerprint). In addition, PFGE is commonly used to assess bacterial strain relatedness in the setting of outbreak investigations when investigators are attempting to determine if multiple bacterial isolates arise from a common source.[26]

DENSITOMETRY

Densitometry is a specialized form of spectrophotometry used to evaluate electrophoretic patterns. Densitometers can perform measurements in an absorbance optical mode and a fluorescence mode, depending on the type of staining of the electrophoretic pattern. An absorbance optical system consists of a light source, filter system, a movable carriage to scan the electrophoretic medium, an optical system, and a photodetector (silicon photocell) to detect light in the absorbance mode. When a densitometer is operated in the absorbance mode, an electrophoretic pattern located on the carriage system is moved across a focused beam of incident light. After the light passes through the pattern, it is converted to an electronic signal by the photocell to indicate the amount of light absorbed by the pattern. The absorbance is proportional to the sample concentration. The filter system provides a narrow band of visible light to provide better sensitivity and resolution of the different densities. This mode of operation is commonly used to evaluate hemoglobin and protein electrophoresis patterns and applications of molecular diagnostics (MDx), including one- and two-dimensional, DNA, RNA, and polymerase chain reaction (PCR) gel electrophoresis bands; dot blots; slot blots; image analysis; and chromosome analysis.

The fluorescence method is used in the case of electrophoretic patterns that fluoresce when radiated by UV light

(340 nm). Densitometers used in this mode include a UV light source and a photomultiplier tube instead of the silicon photocell. When the pattern located on the carriage moves across a focused beam of UV light, the pattern absorbs the light and emits visible light. The light is focused by a collection of lenses onto a UV blocking filter and then to a photomultiplier tube where the visible light is converted into an electronic signal that is proportional to the intensity of the light. In each case, the electrophoretic patterns are evaluated by comparison of peak heights or peak areas of the sample and the standards. Current densitometry systems employ sophisticated software to provide analysis of the signal intensities with high resolution and sensitivity.[9]

CHROMATOGRAPHY

Chromatography is another method used primarily for separation and identification of various compounds. In this procedure, components (solutes) from a mixture are separated by the differential distribution between mobile and stationary phases. In routine clinical practice, paper chromatography has been replaced by three other types of chromatography: thin layer chromatography (TLC), gas chromatography (GC), and high-performance (or pressure) liquid chromatography (HPLC). Chromatographic assays require more time for specimen preparation and performance; they are usually performed only when another assay type is not available or when interferences are suspected with an immunoassay. Chromatographic assays do not require premanufactured antibodies and, therefore, afford better flexibility than an immunoassay.

Thin Layer Chromatography

Thin layer chromatography (TLC) is commonly used for drug screening and analysis of clinically important substances such as oligosaccharides and glycosaminoglycans (e.g., dermatan sulfate, heparin sulfate, and chondroitin sulfate). In this method, a thin layer of gel (sorbent) is applied to glass or plastic, forming the stationary phase. The sorbent may be composed of silica, alumina, polyacrylamide, or starch. The choice of sorbent depends on the specific application because compounds have different relative affinities for the solvent (mobile phase) and the stationary phase. These factors affect the separation of a mixture into the different components. Silica gel is the most commonly used sorbent as it may be used to separate a broad range of compounds, including amino acids, alkaloids, sugars, fatty acids, lipids, and steroids.

Used for identification and separation of multiple components of a sample in a single step, TLC is also used in initial component separation prior to analysis by another technique. Quantification of various substances is possible with TLC; each spot can be scraped off and analyzed individually.[9] Although TLC is a useful screening technique, it has lower sensitivity and resolution than either gas or high-performance chromatography. Another disadvantage, as with gas and high-performance chromatography, is that someone with skill and expertise must interpret the results.

Gas Chromatography

Gas chromatography (GC) is a subtype of column chromatography. This technique is used to identify and quantify volatile substances, such as alcohols, steroids, and drugs in the picogram range (Table 2-1). This technique is also based on the principles of paper and TLC, but it has better sensitivity. Instead of a solvent, GC uses an inert gas (e.g., nitrogen or helium) as a carrier in the mobile phase for the volatile substance being analyzed.

A column packed with inert material, coated with a thin layer of a liquid phase, is substituted for paper or gel. The sample is injected into the column (contained in a heated compartment) where it is immediately volatilized and picked up by the carrier gas. Heating at precise temperature gradients is essential for good separation of the analytes. The gas carries the sample through the column where it contacts the liquid phase, which has a high boiling point. Analytes with lower boiling points migrate faster than those with higher boiling points, thus fractionating the sample components. When the sample leaves the column, it is exposed to a detector. The most common detector consists of a hydrogen flame with a platinum loop mounted above it. When the sample is exposed to the flame, ions collect on the platinum loop and generate a small current. This current is amplified by an electrometer, and the signal is sent on to an integrator or recorder. The recorder produces a chromatogram with various peaks being recorded at different times. Because each sample component is retained for a different length of time, the peak produced at a particular retention time is characteristic for a specific component (**Figure 2-3**). The amount

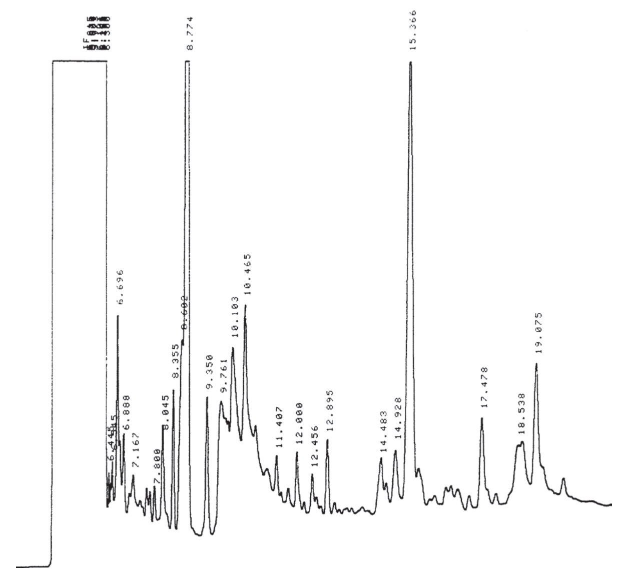


FIGURE 2-3. Gas chromatogram. Area under the curve or peak height of an analyte (e.g., drug or toxin) is compared to the area under the curve or peak height of an internal standard, and then the ratio is calculated. This ratio is compared to a standard curve of peak area ratios to give the concentration of the analyte.

of each component present is determined by the area of the characteristic peak or by the ratio of the peak heights calibrated against a standard curve.

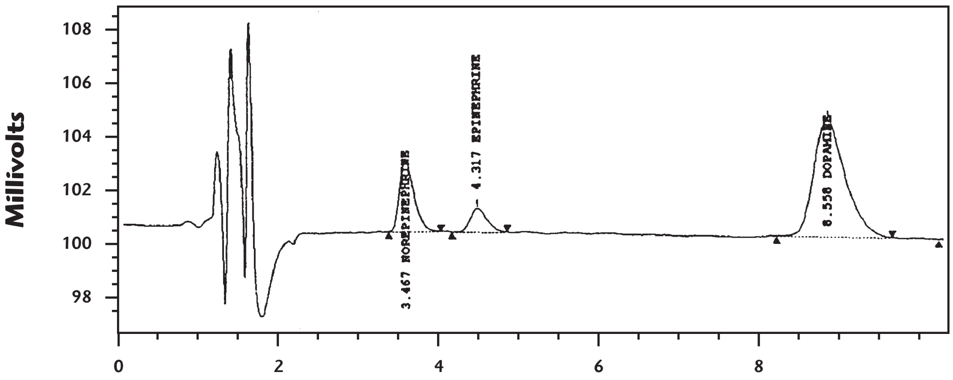
This technique has many advantages, including high sensitivity and specificity. However, it requires sophisticated and expensive equipment. In addition, one or more compounds may produce peaks with the same retention time as the analyte of interest. In cases of such interference, the temperature and composition of the liquid phase can be adjusted for better peak resolution.

High-Performance Liquid Chromatography

High-performance liquid chromatography (HPLC) is widely used, especially in forensic laboratories for toxicologic screening and to measure various drugs (Table 2-2). This is the most widely utilized form of liquid chromatography in clinical laboratories. Its basic principles are similar to those of GC, but it is useful for nonvolatile or heat-sensitive substances.

Instead of gas, HPLC utilizes a liquid solvent (mobile phase) and a column packed with a stationary phase, usually with a porous silica base. The mobile phase is pumped through the column under high pressure to decrease the assay time. The sample is injected onto the column at one end and migrates to the other end in the mobile phase. Various components move at different rates, depending on their solubility characteristics and the amount of time spent in the solid versus liquid phases. As the mobile phase leaves the column, it passes through a detector that produces a peak proportional to the concentration of each sample component. The detector is usually a spectrophotometer with variable wavelength capability in the UV and visible ranges. A signal from the detector is sent to a recorder or integrator, which plots peaks for each component as it elutes from the column (**Figure 2-4**). Each component has its own characteristic retention time so each peak represents a specific component. As with GC, interferences may occur with compounds of similar structure or solubility characteristics; the peaks may fall on top of each other. Better resolution can be obtained by using a column packing with different characteristics or by changing the composition and pH of the mobile phase. Compounds are identified by their retention times and quantified either by computing the area of the peak or by comparing the peak height or area to an internal standard to obtain a peak height or peak area ratio. This ratio is then used to calculate a concentration by comparison to a predetermined standard curve.

Although HPLC offers both high sensitivity and specificity, it requires specialized equipment and personnel. Furthermore, because the substance being determined is usually in a body fluid (e.g., urine or serum), one or more extraction steps are needed to isolate it. Another concern is that because many assays require a mobile phase composed of volatile and possibly toxic solvents, Occupational Safety and Health Administration guidelines must be followed. In addition, assays developed for commercial use may be costly as modifications to published methods are almost always required.



		Peak Results	
Drug	**Retention Time (min)**	**Area (mV sec)**	**Height (mV)**
Norepinephrine	3.467	31,479	2612
Epinephrine	4.317	11,761	847
Dopamine	8.558	112,271	4249

FIGURE 2-4. HPLC chromatogram. Appearance of this chromatogram is similar to the gas chromatogram, and area or peak height ratio is used to quantify the analyte in a sample.

IMMUNOASSAYS

Immunoassays are based on a reaction between an antigenic determinant (i.e., hapten) and a labeled antibody.[27] The label may consist of a radioisotope, an enzyme, enzyme substrate, a fluorophore, or a chromophore. The reaction may be measured by several detection methods including liquid scintillation, UV absorbance, fluorescence, fluorescent polarization, and turbidimetry or nephelometry. The immunoassay method is commonly used for determination of drug concentrations in serum.

Immunoassays can be divided into two general categories: *heterogeneous* and *homogeneous*. In heterogeneous assays, the free and bound portions of the determinant must be separated before either or both portions can be assayed. This separation can be accomplished by various methods including protein precipitation, double antibody technique, adsorption of free drug, and removal by immobilized antibody on a solid phase support. Homogeneous assays do not require a separation step and, therefore, can be easily automated. The binding of the labeled hapten to the antibody alters its signal in a way (color change or reduction in enzymatic activity) that can then be used to measure the analyte concentration.

Early immunoassays used polyclonal antibodies (pAbs), generated as a result of an animal's natural immune response. Typically, an antigen is injected into an animal. The animal's immune system then recognizes the material as foreign and produces antibodies against it. These antibodies are then isolated from the blood. Many different antibodies may be generated in response to a single antigen. The numbers as well as the specificities of the antibodies depend on the size and number of antigenic sites on the antigen. In general, the larger and more complex the antigen (e.g., cell or protein), the more antigenic sites (epitopes) it has and the greater the variety of antibodies formed.

Although pAbs have been used successfully, both specificity and response may vary greatly because of their heterogeneous nature. The result is a high degree of cross-reactivity with similar substances. This cross-reactivity difficulty was eliminated with the development of monoclonal antibodies (moAbs). Prior to 1975, the only moAbs available were from patients suffering from multiple myeloma, a cancer of the blood and bone marrow in which uncontrolled numbers of malignant plasma cells are produced. Usually, these tumor cells produce a single (monoclonal) type of antibody. In 1975, a technique was developed to make moAbs in the laboratory.[28] The technique is based on the fusion of (1) genetic material from plasma cells that produce an antibody but cannot reproduce, and (2) myeloma cells that do not produce an antibody but can reproduce limitlessly. The plasma cells and myeloma cells are cultured together, resulting in a mixture of both parent cells and hybrid cells. This hybrid cell produces the specific antibody and reproduces indefinitely. The mixture is incubated in a special medium, which kills the parent cells and leaves only the hybrid antibody-producing cells alive. The hybrid cells can then be grown using conventional cell culture techniques, resulting in large amounts of the moAb. The development of moAbs has allowed for high sensitivity and specificity in immunoassay technology.

Radioimmunoassay

Today *radioimmunoassay* (RIA) is rarely used in the clinical laboratory and is discussed from a historical perspective. A heterogeneous immunoassay, RIA, was developed in the late 1950s and has been primarily used for endocrinology testing purposes.[29] This technique takes advantage of the fact that certain atoms can be either incorporated directly into the analyte's structure or attached to antibodies.

The primary atoms used in the clinical laboratory fall into two classes: γ-*emitters* and β-*emitters*. The γ-emitters (^{125}I and ^{57}Co) are generally incorporated into compounds such as thyroid hormone and cyanocobalamin (vitamin B_{12}).[13] These types of isotopes can be counted directly with standard γ-counters that utilize a sodium iodide–thallium crystal. When the γ-ray hits the crystal, it gives off a flash of light. This light, in turn, stimulates a photomultiplier tube to amplify the signal. The β-emitters (^{14}C and ^{3}H) are primarily used to measure steroid concentrations.[6] Because endogenous substances tend to absorb the radiation, β-rays cannot be counted directly. Therefore, this technique requires a scintillation cocktail with an organic compound capable of absorbing the β-radiation and reemitting it as a flash of light. This light is then amplified by a photomultiplier tube and counted.

Extremely sensitive, RIA has been made more specific with the introduction of moAbs. Unfortunately, this technique also has several significant disadvantages[13]: a short shelf-life for labeled reagents; lead shielding; waste disposal; monitoring of personnel for radiation exposure; strict record keeping; and special licensing. Because enzyme-linked immunoassays have none of these problems and can perform essentially the same tests as RIA, the clinical use of RIA has decreased in recent years.

Agglutination

The simplest immunoassay is *agglutination*. Typical tests that can be performed using this assay include tests for human chorionic gonadotropin, rheumatoid factor, antigens from infectious agents, such as bacteria and fungi, and antinuclear antibodies. The agglutination reaction, used to detect either antigens or antibodies, results when multivalent antibodies bind to antigens with more than one binding site. This reaction occurs through the formation of cross linkages between antigen and antibody particles. When enough complexes form, clumping results, and a visible mass is formed (**Figure 2-5**). Because the reaction depends on the number of binding sites on the antibody, the greater the number the better the reaction. For example, IgM produces better agglutination than IgG because the former has more binding sites.

The agglutination reaction is also affected by other factors[27]: avidity and affinity of the antibody; n umber of binding sites on the antigen as well as the antibody; relative concentrations

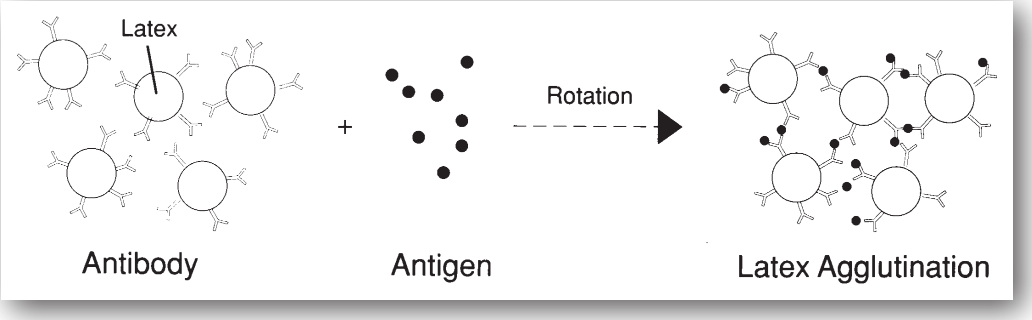


FIGURE 2-5. Schematic of latex agglutination immunoassay. The specimen (cerebrospinal fluid, serum, etc.) contains the analyte (in this case, antigens to bacteria) that causes an easily readable reaction. *Source*: Adapted, with permission, from Power DA, McCuen PJ, eds. Manual of BBI products and laboratory procedures. Cockeysville, MD: Becton Dickinson Microbiology Systems; 1998:77. Courtesy and © Becton, Dickinson, and Company.

of the antigen and antibody; Z-potential (electrostatic interaction that causes particles in solution to repel each other); and viscosity of medium. The two types of agglutination reactions are direct and indirect. *Direct agglutination* occurs when the antigen and antibody are mixed together, resulting in visible clumping. An example of this reaction is the test for *Salmonella typhi* antibody. *Indirect agglutination* (also known as *passive* or *particle agglutination*) uses a carrier for either the antibody or antigen. Originally, erythrocytes were selected as the carrier (as described for hemolytic anemia tests). However, latex-coated particles are now commonly used, and the latex agglutination method is simpler and less expensive than the erythrocyte immunoassay. In addition, latex particles allow titration of the amount of antibody bound to the latex particle, thus reducing variability. Other advantages include a rapid performance time with no separation step, allowing full automation. Disadvantages include expensive equipment and lower sensitivity than either RIA or EIA. The use of an automated particle counter increases the sensitivity of the test 10–1000 times.[30]

Enzyme Immunoassays

Enzyme immunoassays (EIAs) employ enzymes as labels for specific analytes. When antibodies bind to the antigen-enzyme complex, a defined reaction occurs (e.g., color change, fluorescence, radioactivity, or altered activity). This altered enzyme activity is used to quantitate the analyte. The advantages of EIAs include commercial availability at a relatively low cost, long shelf life, good sensitivity, automation, and none of the specific requirements mentioned for RIA.

Enzyme-Linked Immunosorbent Assay

Enzyme-linked immunosorbent assay (ELISA) is a heterogeneous EIA. This assay employs the same basic principles as RIA except that enzyme activity rather than radioactivity is measured. The ELISA is commonly used to determine antibodies directed against a wide range of antigens such as rheumatoid factor, hepatitis B antigen, and bacterial and viral antigens in the serum (Table 2-2).

In a competitive ELISA assay, the specific antibody is adsorbed to a solid phase. Enzyme-labeled antigen is incubated together with the sample containing unlabeled antigen and the antibodies attached to the solid phase. After a specified time, equilibrium is reached between the binding of the enzyme-labeled and unlabeled antigens to the solid phase antibody, and the solid phase is washed with buffer. The remaining product is measured with a spectrophotometer or fluorometer. The amount of the reaction product will be inversely proportional to the amount of unlabeled antigen in the sample because an increasing amount of unlabeled antigen will displace enzyme-labeled antigen from antibody binding.

Enzyme-Multiplied Immunoassay

Enzyme-multiplied immunoassay technique (EMIT) is a homogeneous EIA; the enzyme is used as a label for a specific analyte (e.g., a drug). Many drugs commonly assayed using EMIT are also measured by fluorescence polarization immunoassay (FPIA) (e.g., digoxin, quinidine, procainamide, *N*-acetylprocainamide, and aminoglycoside antibiotics) (Table 2-2). With the EMIT assay, the enzyme retains its activity after attaching to the analyte. For example, to determine a drug concentration, an enzyme is conjugated to the drug and incubated with anti-drug antibody.

As shown in **Figure 2-6**, the test drug (D) is covalently bound to an enzyme that retains its activity and acts as a label. When this complex is combined with antidrug antibody, the enzyme is inactivated. If the antibody and enzyme-bound drug are combined with serum that contains unbound drug, competition occurs. Because the amount of antidrug antibody is limited, the free drug in the sample and the enzyme-linked drug compete for binding to the antibody. When the antibody binds to the enzyme-linked drug, enzyme activity is inhibited. The result is that the serum drug concentration is proportional to the amount of active enzyme remaining. Because no separation step is required, this assay has been automated.

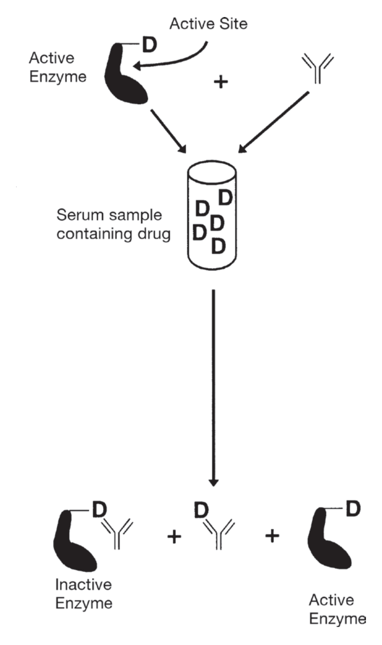


FIGURE 2-6. EMIT. This assay is used in quantifying the drug concentration in a serum sample, as described in the text.

Fluorescent Polarization Immunoassay

Fluorescent polarization immunoassay (FPIA), the most common form of immunoassay, is used to measure concentrations of many serum analytes, such as blood urea nitrogen and creatinine. It is also commonly employed for determining serum drug concentrations of aminoglycoside antibiotics, vancomycin, and theophylline (Table 2-2).

Molecules having a ring structure and a large number of double bonds, such as aromatic compounds, can fluoresce when excited by a specific wavelength of light. These molecules must have a particular orientation with respect to the light source for electrons to be raised to an excited state. When the electrons return to their original lower energy state, some light is reemitted as a flash with a longer wavelength than the exciting light. Fluorescent immunoassays take advantage of this property by conjugating an antibody or analyte to a fluorescent molecule. The concentration can be determined by measuring either the degree of fluorescence or, more commonly, the decrease in the amount of fluorescence present.[13,30] In FPIA, a polarizing filter is placed between the light source and the sample and between the sample and the detector. The first filter assures that the light exciting the molecules is in a particular orientation; the second filter assures that only fluorescent light of the appropriate orientation reaches the detector.

The fluorescent polarization of a small molecule is low because it rotates rapidly and is not in the proper orientation long enough to give off an easily detected signal. To decrease this molecular motion, the molecule is complexed with an antibody. Because this larger complex rotates at a slower rate, it stays in the proper orientation to be excited by the incident light. When unlabeled analyte is mixed with a fixed amount of antibody and fluorescent-labeled analyte, a competitive binding reaction occurs between the labeled and unlabeled analytes. The result is a decrease in fluorescence. Thus, the concentration of unlabeled analyte is inversely proportional to the amount of fluorescence.[30]

Because of their simplicity, automation, and low cost, assays have been developed with relatively high sensitivity for many drugs (e.g., antiepileptics, antiarrhythmics, and antibiotics). The primary difficulty is interference from endogenous substances (lipids and bilirubin) or metabolites of the drugs within the patient specimen.

MASS SPECTROMETRY

Mass spectrometry (MS) involves the fragmentation and ionization of molecules in the gas phase according to their mass to charge ratio (m/z). The resulting mass fragments are displayed on a mass spectrum, or a bar graph, that plots the relative abundance of an ion versus its m/z ratio. Because the mass spectrum is characteristic of the parent molecule, an unknown molecule can be identified by comparing its mass spectrum with a library of known spectra.

A wide array of MS systems has been developed to meet the increasing demands of the biomedical field. However, the basic principles and components of mass spectrometers are essentially the same. These include an inlet unit, an ion source, a mass analyzer, an ion detector, and a data/recording system. Compounds introduced into a mass spectrometer must first be isolated. This is accomplished with separation techniques such as GC, liquid chromatography, and CE, which are used in tandem with mass spectrometers. In a GC/MS system, an interface between the GC and MS components that restricts the gas flow from the GC column into the mass spectrometer is required to prevent a mismatch in the operating pressures between the two instruments. The unit must also be heated to maintain the volatile compounds in the vapor state and remove most of the carrier gas from the GC effluent entering the ion source unit.[31]

Ionization Methods

The ionization of the molecules introduced into MS is accomplished by several methods. In each case, the ion sources are maintained at high temperatures and high vacuum conditions necessary for ionizing vaporized molecules. The *electron ionization* (EI) method, a form of gas-phase ionization, consists of a beam of high-energy electrons that bombard the incoming gas molecules. The energy used is sufficiently high to not only ionize the gas molecules, but also cause them to fragment through the breaking of their chemical bonds. This process yields ion fragments in addition to intact molecular ions that appear in the mass spectra. The EI method is most useful for low molecular weight compounds (<400 daltons) because of problems with excessive fragmentation and thermal decomposition of large molecules during vaporization.[32,33] Therefore, EI is typically used in GC/MS systems that are suitable for applications including the analysis of synthetic organic chemicals, hydrocarbons, pharmaceutical compounds, organic acids, and drugs of abuse.

Chemical ionization (CI) is another form of gas-phase ionization. Because the sample molecule is ionized by a reagent such as methane or ammonia that is first ionized by an electron beam, CI is a less energetic technique than EI. Less fragmentation is produced by this method making it useful for determining the molecular weights of many organic compounds and for enhancing the abundance of intact molecular ions.

Electrospray ionization (ESI), a form of atmospheric pressure ionization, generates ions directly from solution permitting it to be used in combination with HPLC and CE systems. This method involves the creation of a fine spray in the presence of a strong electric field. As the droplets become declustered, the force of the surface tension of the droplet is overcome by the mutual repulsion of like charges, allowing the ions to leave the droplet and enter the mass analyzer. This technique will yield multiple ionic species especially for high molecular weight ions that have a large distribution of charge states, thus making this a very sensitive technique for small, large, and labile molecules.[34] This ionization method is well-suited for the analysis of peptides, proteins, carbohydrates, DNA fragments, and lipids. Other common ionization techniques include *fast atom bombardment* (FAB), which uses high velocity atoms such as argon to ionize molecules in a liquid or solid, and *matrix-assisted laser desorption/ionization* (MALDI), which uses high energy photons to ionize molecules embedded on a solid organic matrix.[34]

Mass Analyzers

Following ionization, the gas phase ions enter the *mass analyzer*. This component of the mass spectrometer separates the ions by their m/z ratios. Commonly used mass analyzers include the double-focusing magnetic sector analyzer, quadrupole mass spectrometers, quadrupole ion trap mass spectrometers, and tandem mass spectrometers.

The *double-focusing magnetic sector* analyzer uses a magnetic field perpendicular to the direction of the ion motion to deflect the ions into a circular path with a radius dependent on the m/z ratio and the velocity of the ion. The detector will then separate the ions by their m/z ratios. However, because the kinetic energy (or velocity) of the molecules leaving the ion source is not necessarily constant, the path radii will become dependent on the velocity and the m/z ratio. To enhance the resolution, an electrostatic analyzer or electric sector is used to allow molecules with only a specific kinetic energy to pass through its field. That is, for a particular kinetic energy, the radius of curvature is directly related to the m/z ratio. This type of analyzer is commonly used in combination with EI and FAB ionization systems.

Quadrupole mass spectrometers act as a filter for molecules or fragments with a specific m/z ratio. This is accomplished by using four, equally spaced parallel rods with direct current (DC) and radio frequency (RF) potentials on opposing rods of the quadrupole. The field produced is along the x- and y-axis. The RF oscillation causes the ions to be attracted or repelled by the rods. Only ions with a specific m/z ratio will have a trajectory along the z-axis, allowing them to pass to the detector, while others will be trapped by the rods of the quadrupole. By varying the RF field, other m/z ranges are selected, thus resulting in the mass spectrum.[33] The quadrupole mass spectrometer, commonly combined with the EI ionization system, is perhaps the most commonly used type of mass spectrometer because of its relatively low cost, ability to analyze m/z ratios up to 3000, and its compatibility with ESI ionization systems.

The *ion trap analyzer* is another form of a quadrupole mass spectrometer, consisting of a ring electrode to which an RF voltage is applied to two end caps at ground potential. This arrangement generates a quadrupole field trapping ions that are injected into the chamber or are generated within it. As the RF field is scanned, ions with specific and successive m/z ratios are ejected from the trap to the ion detector through holes in the caps.[33] The quadrupole ion trap mass spectrometer is notable for its high sensitivity and compact size.

Tandem mass spectrometers use multiple stages of mass analysis on subsequent generations of ion fragments. This is accomplished by preselecting an ion from the first analysis and colliding it with an inert gas, such as argon or helium, to induce further fragmentation of the ion. The next stage involves analysis of the fragments generated by an earlier stage. The abbreviation MS^n is applied to the stages, which analyze fragments beyond the initial ions (MS) to the first generation of ion fragments (MS^2) and subsequent generations (MS^3, MS^4, etc.). These techniques can be tandem in space (two or more instruments) or tandem in time. In the former case, many combinations have been used for this type of analysis. In the later cases, quadrupole ion trap devices are often used and can achieve multiple MS^n measurements.[35] Tandem mass analysis is primarily used to obtain structural information such as peptides sequences, small DNA/RNA oligomers, fatty acids, and oligosaccharides. Other mass analyzers, such as time-of-flight and Fourier transform mass spectrometers, are not commonly used for clinical applications.

Ion Detector

The *ion detector* is the final element of the mass spectrometer. Once an ion passes through the mass analyzer, a signal is produced in the detector. The detector consists of an electron multiplier that converts the energy of the ion into a cascade of secondary electrons (similar to a photomultiplier tube), resulting in about a million-fold amplification of the signal. Due to the rapid rate at which data are generated, computerized data systems are indispensable components of all modern mass spectrometers. The introduction of rapid processors, large storage capacities, and spectra databases has lead to automated high throughput. Miniaturization of components has also led to the development of bench-top systems practical for routine clinical laboratory analysis. Clinical applications include newborn screening for metabolic disorders, hemoglobin analysis, drug testing, and microbial identification. Pharmaceutical applications include drug discovery, pharmacokinetics, and drug metabolism. Clinical microbiology has seen a radical shift in the last decade in the approach used to identify microorganisms. Traditional identification had been based on biochemical testing and relies on well-isolated colonies from microbiologic media for testing. These methods are time-consuming, and much progress has been made to reduce the turnaround time to provide clinicians with more rapid identification to provide more targeted antimicrobial chemotherapy. Some laboratories have adopted the use of MALDI-TOF (time of flight) to provide more rapid bacterial or yeast identification of clinical pathogens.

In this application, a colony from an agar plate or directly from a positive blood culture bottle is applied to a card and is overlaid with a matrix and allowed to dry on the plate. The plate is loaded onto the analyzer where a laser ionizes the sample. The mass of the ions generated from the ionization process is analyzed using a flight tube, which detects the lighter ions that travel faster than the heavier, slower traveling ions. The result of the detection of the ionized targets (typically bacterial ribosomal proteins) is the generation of a unique mass spectrum. In this spectrum, the mass-to-charge ratio is plotted against the signal intensity. Therefore, the system only detects highly abundant proteins that are of low mass and readily ionized by the laser. In effect, the mass profile generated is a bacterial fingerprint. The spectrum is compared to a comprehensive database of spectra for well-characterized bacterial or fungal pathogens. Depending on the similarity of the peaks, laboratories can accurately identify a colony to the genus or species level.[36]

CYTOMETRY

Cytometry is defined as a process of measuring physical, chemical, or other characteristics of (usually) cells or other biological particles. Although this definition encompasses the fields of flow cytometry and cellular image analysis, many additional methods are now used to study the vast spectrum of cellular properties. Consequently, the term *cytomics* has been introduced. Cytomics is defined as the science of cell-based analysis that integrates genomics and proteomics with dynamic functions of cells and tissues. The technology used includes techniques discussed in this chapter, such as flow cytometry and MS, and others that are beyond the scope of this chapter.

Flow Cytometry

Flow cytometry is the technology used to measure properties of cells as they move or flow in liquid suspension.[37] It is a technique used to measure multiple characteristics of individual cells within heterogeneous populations. Instruments generally referred to as *flow cytometers* are based on the principles of laser-induced fluorometry and light scatter. The terminology can become confusing as various conventions have taken root over the years. However, regardless of the principles of detection or measurement, the term *flow cytometry* may in general be applied to technologies that rely on cells moving in a fluid stream for analysis.

The hematology analyzer, an instrument employing flow cytometry, also incorporates the principles of impedance, absorbance, and laser light scatter to measure cell properties and generate a complete blood count laboratory report. The basis of cell counting and sizing in hematology analyzers is the Coulter principle, which relates counting and sizing of particles to changes in electrical impedance across an aperture in a conductive medium (created when a particle or cell moves through it). The basic system consists of a smaller chamber within a larger chamber, both filled with a conductive medium and each with one electrode across in which a constant DC is applied. The fluids within each chamber communicate through a small aperture (100 μm) or sensing zone. When a nonconductive particle or cell passes through the aperture, it displaces an equivalent volume of conductive fluid. This increases the conductance and creates a voltage pulse for each cell counted, the intensity of which is proportional to the cell volume.[16]

In hematology analyzers, blood is separated into two samples for measurement. One volume is mixed with a diluent and delivered to a chamber where platelet and erythrocyte counts are performed. Particles with volumes between 2 and 20 femtoliter (fL) are counted as platelets, and particles with volumes >36 fL are counted as erythrocytes. The other volume is mixed with a diluent, and an erythrocyte lysing reagent is used to permit leukocyte (>36 fL) counts to be performed. The number of cells in this size range may be subtracted from the erythrocyte count performed in the other chamber.

Modern hematology analyzers employ additional technologies to enhance the resolution of blood cell analysis. The RF energy is used to assess important information about the internal structure of cells such as nuclear volume. Laser light scatter is used to obtain information about cell shape and granularity. The combination of these and other technologies—such as light absorbance for hemoglobin measurements—provide accurate blood cell differentials, counts, and other important blood cell indices. These basic principles are common to many hematology analyzers used in clinical laboratories. However, each uses different proprietary detection, measurement and software systems, and ways of displaying the data.

Flow cytometers incorporate the principles of fluorometry and light scatter to the analysis of particles or cells that pass within a fluid stream. This technology provides multiparametric measurements of intrinsic and extrinsic properties of cells. Intrinsic properties, including cell size and cytoplasmic complexity, are properties that can be assessed directly by light scatter and do not require the use of any type of probe. Extrinsic cellular properties, such as cell surface or cytoplasmic antigens, enzymes or other proteins, and DNA/RNA, require the use of a fluorescent dye or probe to label the components of interest and a laser to induce the fluorescence (older systems used mercury arc lamps as a light source) to be detected.

The basic flow cytometer consists of four types of components: fluidics, optics, electronics, and data analysis. Fluidics refers to the apparatus that directs the cells in suspension to the flow cell where they will be interrogated by the laser light. Fluidics systems use a combination of air pressure and vacuum to create the conditions that allow the cells to pass through the flow chamber in single file. The optical components include the laser (or other light source), flow chamber, monochromatic filters, dichroic mirrors, and lenses. These are used to direct the scattered or fluorescent light to detectors, which measure the signals that are subsequently analyzed.[37]

The light scattered by the cell when it reaches the flow chamber is used to measure its intrinsic properties. *Forward-scattered light* (FSC) is detected by a diode and reflects the size of the passing cell. *Side-scattered light* (SSC) is detected by a photomultiplier tube at an angle approximately 90 degrees to the laser beam. The SSC is a function of the cytoplasmic complexity of the cell, including the granularity of the cell. The correlated measurements and analysis of FSC and SSC can allow for differentiation among cell types (i.e., leukocytes) and depicted on a scattergram.

The analysis of extrinsic properties is more complicated. The measurement of DNA or RNA, for example, requires the use of intercalating nucleic acid dyes such as propidium iodide. The detection of antigenic determinants on cells can be performed with fluorescent-labeled moAbs directed at these antigens. In each case, the principle of detection involves the use of laser light to excite the fluorescent dye and detect its emitted signal. Fluorescent dyes are characterized by their excitation (absorption) and emission wavelength spectra and by the difference between the maxima of these spectra or Stokes shift (discussed in the spectrophotometry section). These properties permit the use of multiple fluorescent probes on a single cell.

To illustrate the operation of a flow cytometer, consider a four-color, six-parameter (FSC and SSC) configuration (**Figure 2-7**).[38] An argon gas laser with a wavelength of 488 nm is commonly used because it simultaneously excites several different dyes that possess different emission wavelengths. Fluorochromes conjugated with moAbs that may be used include fluorescein isothiocyanate, phycoerythrin (PE), energy-coupled dye, and Cy5PE (tandem dye composed of the carbocyanine derivative Cy5 and PE) with peak emission wavelengths of approximately 520, 578, 613, and 670 nm, respectively. The emitted light at each of these wavelengths is detected at an angle of 90 degrees. The array of optical filters selects light in each wavelength region and directs it to a different photomultiplier tube where it is detected, amplified, and converted into an electronic signal. This measurement can be made on thousands of cells in a matter of seconds. The result is a histogram that identifies distinct cell populations based on light scatter and extrinsic properties. In the case of blood, a histogram will distinguish lymphocytes, monocytes, and granulocytes by light scatter. The B cell, T cell, T-cell subsets, and natural killer cell populations can all be distinguished.

This important method of cell analysis has found many applications in medicine making it a relatively common

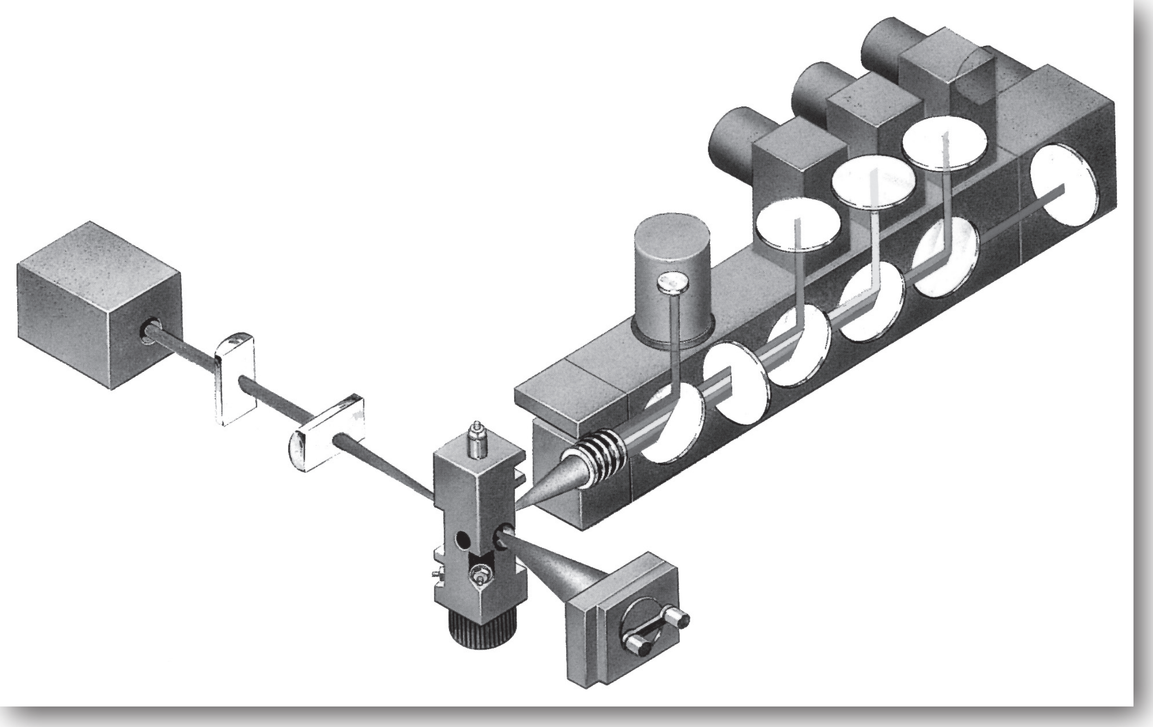


FIGURE 2-7. Schematic of a four-color flow cytometry system. The laser beam is focused onto the flow cell through which the cell suspension is directed. Scattered light is detected by the forward and side scatter detectors. Emitted light from specific moAb labeled with fluorochromes are detected. Appropriate dichroic long pass filters direct the specific wavelength of light through a narrow band pass filter and then to the appropriate PMT (provided courtesy of Beckman Coulter).

clinical laboratory instrument. Flow cytometry analysis is routinely used to assist in classifying the type of leukemia and lymphoma, derive prognostic information in these and other malignancies, monitor immunodeficiency disease states such as HIV/AIDS, enumerate stem cells by cluster differentiation (CD34), and assess various functional properties of cells.

Image Cytometry

Image cytometry, more commonly known as *histology*, is a laboratory method that uses instruments and techniques to analyze tissue specimens. Examining individual cells, rather than the collection of cells that make up a tissue, is referred to as *cytology*. The basic components of an image cytometry system may include a microscope, camera, computer, and monitor. Variations and complexity of these systems exist, which are beyond the scope of this chapter. However, the essence of these instruments is the ability to acquire images in two or three (confocal microscopy) dimensions to study the distribution of various components within cells or tissues. The high optical resolution of these systems is an important determinant in obtaining morphometric information and precise data about cell and tissue constituents through the use of fluorescence/absorbance-based probes, as in flow cytometry.[39] Specific applications of image cytometry generally involve unique methods of cell or tissue preparation and other modifications. This lends to the versatility of this technology, which yields such applications as the measurement of DNA content in nuclei to assess prognosis in cancer and the detection of specific nucleic acid sequences to diagnose genetic disorders.

In Situ Hybridization

Among the methods of image cytometry, *in situ hybridization* is perhaps the most commonly used in the clinical laboratory, particularly in molecular cytogenetics laboratories. In situ hybridization is used to localize nucleic acid sequences (entire chromosomes or parts, including genes) in cells or tissues through the use of probes, which consist of a nucleic acid sequence that is complementary to the target sequence and labeled in some way that makes the hybridized sequence detectable. These principles are common to all methods of in situ hybridization, but they differ in the type of probe that is used. Fluorescent probes, which provide excellent spatial resolution, have become a preferred method of in situ hybridization for many applications. (Radioactive probes are also used for this application. However, because their spatial resolution is limited, detection and artifacts are often produced.)

Fluorescent in situ hybridization (FISH) is a powerful molecular cytogenetics technique used for detecting genes and genetic anomalies and monitoring different diseases at the genetic level. These assays are more sensitive and can detect chromosomal abnormalities that cannot be appreciated by routine chromosome analysis (i.e., karyotyping). Typically, metaphase chromosomes or interphase nuclei are denatured on a slide along with a fluorescent labeled DNA probe. The probe and chromosomes are hybridized, the slide is washed, counterstained, and analyzed by fluorescent microscopy. There are various types of FISH probes that can be utilized, such as DNA probes to detect nick translations or RNA probes that can detect in vitro transcription. An appropriate arrangement of filters is used to direct the relevant wavelength of light from the light source to excite the fluorescent molecule on the probe. All but the emission wavelength of light is blocked with a special filter permitting the signal from the probe to be visualized.[40] In molecular cytogenetics, these assays are commonly used to identify gene fusions or translocations.

MOLECULAR DIAGNOSTICS

Molecular diagnostics (MDx) have initially been introduced into the clinical laboratories as manual, labor intensive techniques. This discipline has experienced an overwhelming period of maturation in the past several years. Testing has moved quickly from highly complex, labor intensive procedures to more user-friendly and semiautomated protocols, and the application potential of MDx continues to evolve. Nucleic acid amplification technologies are among the procedures that have most revolutionized MDx testing.

Nucleic Acid Amplification

Polymerase chain reaction (PCR) is the most frequently used of these technologies. Other amplification techniques that are used in clinical laboratory procedures include ligase chain reaction, transcription mediated amplification, branched DNA amplification, and nucleic acid sequence-based amplification.

The PCR technology is used principally for detecting microbiologic organisms and genetic diseases (Table 2-2). Microorganisms identified by this process include chlamydia, cytomegalovirus, Epstein-Barr virus, HIV, mycobacteria, and herpes simplex virus. Although the number of organisms that can be identified for clinical diagnostic purposes is limited at present, this list is growing. Furthermore, PCR can often identify organisms with greater speed and sensitivity than conventional methods. For clinical microbiology laboratories, PCR methods are attractive because they are rapid, sensitive, and specific. Many laboratories have moved from culture-based methods to molecular amplification methods for the rapid identification of patients that may be colonized with multidrug-resistant organisms, such as *Clostridium difficile,* methicillin-resistant *Staphylococcus aureus*, or vancomycin-resistant enterococci. Rapid identification of these patients is crucial in healthcare settings that often place these patients on contact precautions to try and reduce the spread of these organisms. The PCR applications in microbiology can also be used to identify organisms carrying antibiotic resistance genes, such as the *Klebsiella pneumoniae* carbapenemase bla_{KPC} gene that confers resistance to all β-lactam antibiotics among members of the *Enterobacteriaceae* and other gram-negative bacilli.

Genetic diseases diagnosed using PCR include α-1 antitrypsin deficiency, cystic fibrosis, sickle cell anemia, fragile X syndrome, Tay-Sachs disease, drug-induced hemolytic anemia, and Von Willebrand disease. In addition, cancer research has benefited from PCR through the diagnosis of various cancers (e.g., chronic myeloid leukemia and pancreatic and colon cancers) as well as through the detection of residual disease after treatment.[41] This technique is used to amplify specific DNA and RNA sequences enzymatically.

In addition, PCR takes advantage of the normal DNA replication process. In vivo, DNA replicates when the double helix unwinds and the two strands separate. A new strand forms on each separate strand through the coupling of specific base pairs (e.g., adenosine with thymidine and cytosine with guanosine). The PCR cycle is similar and consists of three separate steps (**Figure 2-8**)[30]:

1. Denaturation—the two strands of DNA are thermally separated.
2. Primer annealing—sequence-specific primers are allowed to hybridize to opposite strands flanking the region of interest by decreasing the temperature.
3. Primer extension—DNA polymerase then extends the hybridized primers, generating a copy of the original DNA template.

The efficiency of the extension step can be increased by raising the temperature. Typical temperatures for the three steps are 201.2 °F (94 °C) for denaturation, 122 °F to 149 °F (50 °C to 65 °C) for annealing, and 161.6 °F (72 °C) for extension. Note that cycle temperatures are influenced by the specific enzyme used, the primer sequence, and the genomic sample. Because one cycle is typically completed in less than three minutes, many cycles can occur within a short time, resulting in the exponential production of millions of copies of the target sequence.[42] The genetic material is then identified by agarose gel electrophoresis.

One potential disadvantage of this method is contamination of the amplification reaction with products of a previous PCR (carryover), exogenous DNA, or other cellular material. Contamination can be reduced by prealiquoting reagents, using dedicated positive-displacement pipettes, and physically separating the reaction preparation from the area where the product is analyzed. In addition, multiple negative controls are necessary to monitor for contamination. Also common in clinical laboratories are instrument platforms that can perform real-time (q)PCR as well as multiplex PCR, which allows amplification of two or more products in parallel in a singly reaction tube.[42] Several in vitro diagnostic companies such as BD Diagnostics, BioFire, Cepheid, and Nanosphere have Food and Drug Administration (FDA)-approved platforms that can allow for simultaneous detection of multiple microorganism targets. Use of these multiplex assays is attractive because they require minimal sample volumes to generate multiple results.

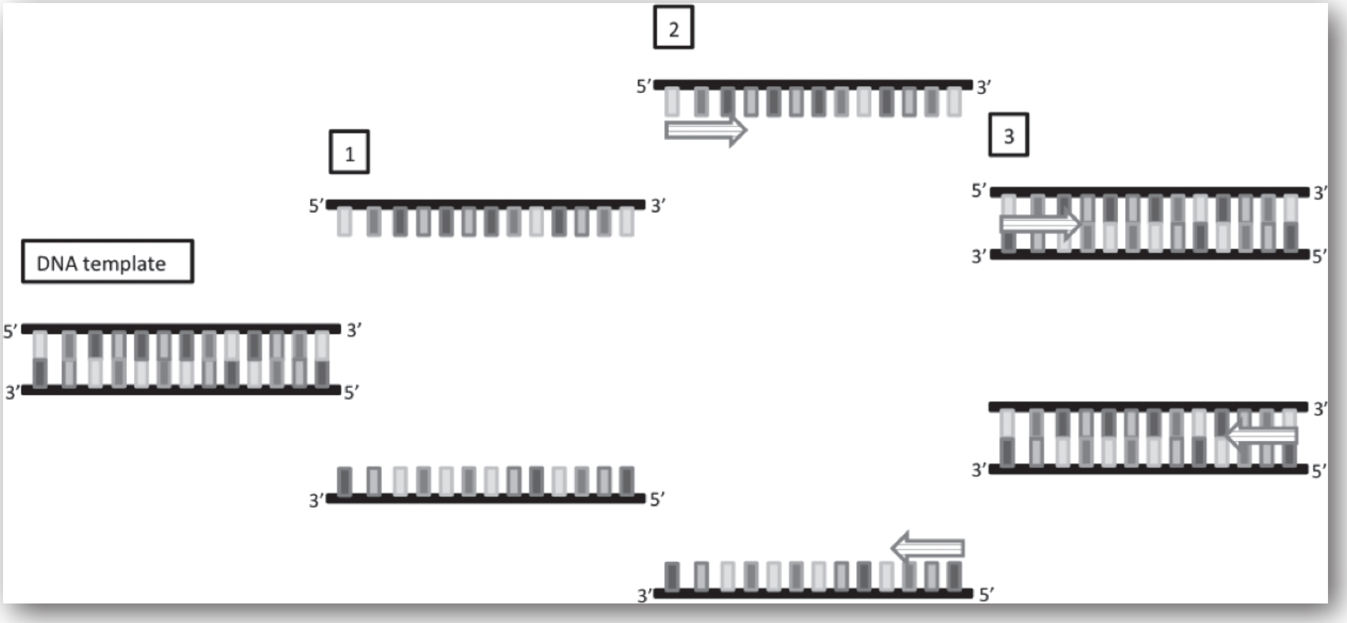


FIGURE 2-8. Elements of a single PCR reaction cycle. Beginning with your DNA template, the sample is added into a microtube with DNA polymerase, forward and reverse sequence specific primers that will bind to and amplify the region of interest within the template, and excess amounts of deoxynucleotide triphosphates (dNTP). During Step 1 (denaturation), the sample is heated between 93–96 °C to separate the double stranded DNA into two single strands. During Step 2 (annealing), the forward and reverse sequence specific primers will bind (anneal) to complementary sequences within the template. Annealing occurs between 50–70 °C. Step (3) is extension of the template, which occurs at 68–75 °C. During extension, DNA polymerase will catalyze the addition of complementary dNTP to the primer using the sample DNA as the template. This completes one cycle of the PCR reaction yielding two copies of the amplified region of interest.

GENOMICS, EPIGENETICS, AND PROTEOMICS

Newly developed techniques capable of examining the DNA, messenger RNA (mRNA), and proteins of cells have provided a framework for detailed molecular classifications and treatments of diseases. Genetic analysis of cystic fibrosis, for example, has shown the disease to be the result of over 1500 different mutations in the gene cystic fibrosis transmembrane conductance regulator.[43] The most common mutation accounts for two thirds of cystic fibrosis cases. Several related developments, especially in the areas of tumor classifications, are based on the fields of genomics, epigenetics, and proteomics. The most important laboratory procedures are array-based comparative genomic hybridization and the data derived from these studies—bioinformatics.

Genomics

The study of all the genes of a cell, its DNA sequences, and the fine-scale mapping of genes is the science of *genomics.* A genome is the sum total of all genes of an individual organism. Knowledge of full genomes has created multiple possibilities, mainly concerned with patterns of gene expression associated with various diseases.[44,45]

Epigenetics

Epigenetics refers to modifications of the genome that are functionally relevant but do not involve a change in the nucleotide sequence. Histone deacetylation and DNA methylation are examples of such changes, both of which serve to suppress gene expression without altering the sequence of the silenced genes. Such changes may continue to exist for many cell divisions and even the remainder of the cell's life, as well as for future generations of cells. However, because there is no change in the underlying DNA sequence of the organism, nongenetic factors cause the organism's genes to express themselves differently.[46]

Proteomics

The study of the full complement of proteins in a cell or tissue is called *proteomics* and includes the comprehensive analysis and characterization of all of the proteins including their structure and function that are encoded by the human genome. Protein-based assays were among the first assays to be approved by the FDA, mostly using immunohistochemistry techniques. Most important biological functions are controlled by signal transduction, which are processes governed by the enzyme activities of proteins. Diseases such as cancer, while fundamentally the result of genomic mutations, manifest as dysfunctional protein signal transduction. Many pharmaceuticals are now being developed to aim at modulating the aberrant protein activity, not the genetic defect.[47-49]

Proteomics will eventually have a great impact in the practice of medicine. Although the genome is the source of basic cellular information, the functional aspects of the cell are controlled by and through proteins, not genes. The main challenge to the study of proteomics is due to the proteome's complexity compared to the genome. The human genome encodes approximately 23,000 genes, about 21,000 of which encode proteins. However, the total number of proteins in human cells is estimated to be between 250,000 to 1 million. Furthermore, proteins are dynamic and constantly undergo changes, synthesis, and breakdown. Currently, most of the FDA-approved targeted therapeutics are directed at proteins and not genes.

ARRAY-BASED COMPARATIVE HYBRIDIZATION

Molecular profiles of cells can now be determined using *array-based comparative hybridization.*[50] This technique is especially useful in profiling tumor cells. Until recently, changes occurring in cancer cells were studied one at a time or in small groups in small sets of tumors. New array comparative hybridization or microarray technology ("gene chips") has enabled investigators to simultaneously detect and quantify the expression of large numbers of genes (potentially all genes) in different tumors using mRNA levels. In this technique, samples are obtained from tissues embedded in paraffin blocks, and serve as the sources to prepare new blocks that may contain up to thousands of tissue fragments. These multiple samples are then used to test the expression of potential tumor markers by mRNA expression profiling. The mRNA levels, however, do not always correspond to changes in tumor cell proteins. The quantity of protein within a cell depends not only on the amount and rate of transcription and translation, but also on protein breakdown and the rate of transport out of the cell. Although tissue used for mRNA profiling may include both tumor and stromal cells, by adding immunohistochemistry methods, specific proteins in tissue sections originating from both normal as well as tumor cells can be identified.

As a specific example, several types of breast cancer cells, which were previously identified only by morphology, are now being studied by array-based comparative hybridization techniques. Combined with immunohistochemistry staining and protein expression levels, new subtypes that were not previously well defined have been identified (e.g., the basal-like carcinomas).[51] As a consequence, new treatment modalities have been developed. Array-based comparative hybridization methods have also identified new subtypes of other tumors, such as lymphomas and prostate cancer with potential for susceptibility and prognosis.[52,53]

NANOTECHNOLOGY

Nanotechnology refers to the emerging science that studies interactions of cellular and molecular components at the most elemental level of biology, typically clusters of atoms, molecules, and molecular fragments. Nanoscale objects have dimensions smaller than 100 nm. At this dimension, smaller than human cells (which vary from 10,000–20,000 nm in diameter), small clusters of molecules and their interactions can be detected. Nanoscale devices smaller than 50 nm can easily enter most cells, while those smaller than 20 nm can move out of blood vessels, offering the possibility that these devices will be able to enter biological chambers, such as the blood–brain barrier or the gastrointestinal epithelium, and identify tumors, abnormalities, and deficiencies in enzymes and cellular receptor sites. Within these biological chambers, they will be able to interact with an individual cell in real time and in that cell's native environment.

Despite their small size, nanoscale devices can also hold tens of thousands of small molecules, such as a magnetic resonance imaging contrast agent or a multicomponent diagnostic system capable of assaying a cell's metabolic state. A good example of this approach will capitalize on existing "lab-on-a-chip" and microarray technologies developed at the micron scale. Widely used in biomedical research and to a lesser extent for clinical diagnostic applications today, these technologies will find new uses when shrunk to nanoscale. (In some instances, nanotechnology has already taken advantage of previous clinically relevant technological developments on larger scales.)

Currently, innovative testing is available for many different viruses, mutation analysis, and hematological and solid tumors. With continuing advances and developments in nanotechnology, it is impossible to speculate as to what this new area of testing holds for the future of the clinical laboratory.

SUMMARY

This chapter presents a brief overview of the more common and some emerging laboratory methodologies, including their potential advantages and pitfalls. Some historical methods have been discussed to provide a basis and description of the simple principles on which the more complex methods are based. A summary of some of the most common assay methods performed for routine laboratory tests is provided in Table 2-2.

Due to its simplicity and improved sensitivity, ISE has replaced flame photometry as the principal method for measuring serum and urine electrolytes in clinical specimens. Some methods, including turbidimetry, nephelometry, and spectrophotometry, are used in conjunction with other tests such as immunoassays. With these methods, concentrations of substances such as immune complexes are able to be determined.

Mass spectrometry is the gold standard for the identification of unknown substances, including drugs of abuse. Many of the newest designer drugs and bath salts are only identifiable based on this technique, as no other methodologies exist to detect them in clinical specimens. The two principal forms of chromatography are liquid and gas. Both types are similar in that they depend on differences in either solubilities or boiling points, respectively, to separate different analytes in a sample. Another group of important tests are the immunoassays: EIA, EMIT, ELISA, and FPIA. All of these methods depend on an immunologically mediated reaction that increases sensitivity and specificity over RIA. These assays are commonly used to determine routine clinical chemistries and drug concentrations. PCR and other nucleic acid amplification techniques are used to amplify specific DNA and RNA sequences, primarily in the areas of microbiology and detection of genetic diseases. Finally, with the potential advances envisioned in the area of nanotechnology, the laboratory will be able to provide clinicians with information and access to the patient's cellular and molecular environments, thus providing the ability to target therapies at the exact site of the pathologic process.

The rapid technological advancement of laboratory instrumentation has led to the implementation of new and enhanced clinical laboratory methodologies, including MS, cytometry, laboratory automation, and point-of-care testing. Although laboratory medicine endeavors to keep pace with the burgeoning developments in biomedical sciences, especially with an increase in the sophistication of the tests, it is essential that today's clinicians have a basic understanding of the more common and esoteric tests to select the most appropriate one in each case. All of these developments will translate directly into improved patient care.

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3

PRIMER ON DRUG INTERFERENCES WITH TEST RESULTS

Mary Lee

OBJECTIVES

After completing this chapter, the reader should be able to

- Distinguish between in vivo and in vitro drug interferences with laboratory tests
- Identify suspected drug–laboratory test interference in a logical, systematic manner given a drug and a laboratory test
- Devise a stepwise process to confirm that a drug is causing a clinically significant drug–laboratory test interference
- Distinguish among tertiary, secondary, and primary literature resources about drug–laboratory test interferences
- Apply a systematic process to search and identify medical literature relevant to a suspected drug–laboratory test interference situation

Through a variety of mechanisms, drugs can interfere with laboratory test results. If the clinician who has ordered the laboratory test is not aware that the drug has altered the results of the test, inappropriate management of the patient may follow including unnecessary hospitalization, extra office visits, or additional laboratory or clinical testing—all of which may increase the cost of healthcare. This chapter addresses this situation and provides resources that health professionals can use to better interpret laboratory tests when a drug is suspected to interfere with test results.

IN VIVO AND IN VITRO DRUG INTERFERENCES WITH LABORATORY TESTS

When a drug interferes with a laboratory test result, it alters the laboratory value. Mechanisms for drug interference of clinical laboratory tests can be classified as either in vivo or in vitro.[1] *In vivo drug interferences* can also be called physiological and can be subclassified as pharmacological or toxicological. In vivo interferences account for most effects of drugs on laboratory tests.[2] In contrast, the term *in vitro interference* is used synonymously with analytical or methodological interference.

In Vivo Interference

An in vivo interference is an actual change in the analyte concentration or activity prior to specimen collection and analysis. The assay measurement is actual and accurate and reflects a change in the measured substance that has occurred in the patient. Therefore, an in vivo interference will always change a laboratory test result, independent of the assay methodology. A drug can produce an in vivo interference in several ways. By a direct extension of its pharmacological effects, a drug can produce changes in some laboratory test results. For example, thiazide and loop diuretics will commonly cause increased renal elimination of potassium. Therefore, decreased serum potassium levels can occur in treated patients. In these patients, hypokalemia is actual and accurate. Similarly, increased blood urea nitrogen (BUN) levels can occur as a result of excessive fluid loss during treatment with thiazide and loop diuretics.

Other drugs produce changes in laboratory test results by producing in vivo toxicological effects. As the drug damages a particular organ system, abnormal laboratory tests may be one of the first signs of the problem. For example, as isoniazid and rifampin produce hepatotoxicity, elevated hepatic transaminases will signal the onset of liver inflammation. Similarly, as a prolonged course of high-dose aminoglycoside antibiotic causes acute proximal tubular necrosis, serum creatinine and serum trough aminoglycoside levels will increase steadily if the antibiotic is not stopped or if the antibiotic dose is not reduced. In the face of cyclophosphamide-induced bone marrow suppression, neutropenia will become evident 10–14 days after the dose has been administered.

In Vitro Interference

Drugs in a patient's body fluid or tissue can directly interfere with a clinical laboratory test during the in vitro analytical process. This type of drug–laboratory test interaction is highly dependent on the laboratory test methodology, as the reaction may occur with one specific assay method but not another. For example, serum digoxin levels are commonly determined using a radioimmunoassay, a fluorescent polarization immunoassay, or a TDx assay. However, these assays are based on the three-dimensional structure of the digoxin molecule, and many other drugs with a similar chemical structure to digoxin (e.g., spironolactone, estrogen replacement products, cortisol, or digoxin-like substances) can cross-react with the assay.[3] A falsely increased or decreased serum digoxin level can result.[3,4] To determine the true serum digoxin level in this situation, another assay technique (e.g., high-pressure liquid chromatography [HPLC]) may be used. A similar problem occurs with fosphenytoin, which cross-reacts with phenytoin when measured with immunoassay methods.[5] In addition, substances that are prepackaged in or added to the in vitro system before or after sample collection can cause laboratory test interference in vitro. As an example, test tubes sometimes contain lithium heparin or sodium fluoride. Heparin can interfere with aminoglycoside assays, and fluoride can cause false increases in BUN when measured by the Ekatchem assay.

Alternatively, a drug may cause discoloration of the body fluid specimen, which may interfere with colorimetric, photometric, or fluorometric laboratory-based assay methods. For example, phenazopyridine causes an orange-red discoloration of urine that may be mistaken for blood. Nitrofurantoin may cause a brown discoloration of the urine that may cause alarm for the patient. These types of drug interference with laboratory testing can be detected visually and appropriate attribution of the abnormality should be made by knowledgeable clinicians and clinical laboratory staff.

Other common mechanisms by which drugs cause in vitro interferences with laboratory tests include the following:

- A drug reacts with reagent to form a chromophore (e.g., cefoxitin or cephalothin) with the Jaffe-based creatinine assay.
- A drug reacts with immunoassay's antibody that is intended to be specific for the analyte. For example, caffeine cross-reacts in the theophylline assay; digitoxin, digoxin metabolites, antigen-binding fragments derived from antidigoxin antibodies (used for treating digoxin intoxication), spironolactone, and canrenone (the major metabolite of spironolactone) cross-react with digoxin immunoassays.[3]
- A drug alters the specimen pH (usually urine) so that reagent reactions are inhibited or enhanced. For example, acetazolamide produces an alkaline urinary pH that causes false-positive proteinuria with reagent dip strips.
- A drug has chemical properties similar to the analyte. For example, patients who receive radiographic contrast media, which contain iodine, may exhibit altered laboratory values for protein-bound iodine.
- A drug chelates with an enzyme activator or reagent used in the in vitro laboratory analysis.
- A drug absorbs at the same wavelength as the analyte. For example, methotrexate interferes with analytic methods using an absorbance range of 340–410 nm.

In addition to the parent drug, other drug-related components may cause significant interferences with laboratory tests. Metabolites can cross-react with the parent drug in an assay, such as in the case with cyclosporine. Its metabolites cross-react with the parent drug in HPLC assays and can produce a falsely high measurement of the concentration of cyclosporine.[6] Contaminants in herbal products, which are subject to less regulation than medications in the United States, may interfere with some laboratory tests.[7] Inactive ingredients of some drug products, which includes excipients such as lactose or starch, preservatives, colorants, or flavoring agents, may influence assay results. Although most manufacturers do report the inactive ingredients in their products, little systematic research has been performed to assess the impact of these substances on laboratory tests. Compounding these factors, many laboratory test interferences are concentration-related, and many drug metabolites and their usual plasma concentrations have yet to be identified. Therefore, systematic study of all of these potential causes of interactions is difficult to conduct and is not available in many cases.[8]

Simultaneous In Vitro and In Vivo Effects

Some drugs can affect an analyte both in vivo and in vitro. In these situations, interpretation is extremely difficult because the degree of impact in each environment cannot be determined easily. For example, when a drug produces hemolysis in a patient with glucose-6-phosphate dehydrogenase deficiency that is exposed inadvertently to ciprofloxacin, hemolytic anemia may result. Hemolyzed red blood cells produce a red discoloration of the plasma or serum. The hemoglobin released from the damaged red blood cells can interfere with analysis of alkaline phosphatase or γ-glutamyl transferase, both of which can be assayed using a spectrophotometric analysis that depends on color changes after a chemical reaction.[8,9] Simultaneous in vitro and in vivo drug interferences with laboratory tests can also occur commonly when drugs increase bilirubin or when a drug causes lipemia.[10]

IDENTIFYING DRUG INTERFERENCES

Incidence of Drug Interferences

The true incidence of drug interferences with laboratory tests is unknown. This is because many situations probably go undetected. However, as the number of laboratory tests and drugs on the U.S. commercial market increase, it is likely that the number of cases of in vivo interferences will also increase. As a reflection of this, consider the number of drug–laboratory test interferences reported by D. S. Young, author of one of the classic literature references on this topic. In the first edition

MINICASE 1

Trying to Get the Dose Right

Samuel M., a 68-year-old, African-American male patient, complains of decreased sexual drive and erectile dysfunction for one year. His wife, who is about 20 years younger, has sent him to the clinic for medical treatment. Samuel M. reports retiring from his job as a mailman about three years ago. Since then, he has kept active by volunteering at a nearby community center and babysitting his grandchildren. He reports no other problems. Samuel M. also has well-controlled essential hypertension, which has been treated with hydrochlorothiazide and enalapril for the past five years. He is sickle cell trait positive. Pertinent findings on physical exam reveals mild gynecomastia, small testicles, and a normal penis.

Samuel M. is suspected of having late-onset hypogonadism, which is confirmed by two separate serum testosterone measurements of 200 ng/dL and 185 ng/dL for three months. Testosterone enanthate 200 mg intramuscularly every two weeks was initiated. At the end of the third month of treatment, his hematocrit is 45%, liver function tests are normal, BUN is 15 mg/dL, serum creatinine is 1.3 mg/dL, lipid profile is normal, and serum testosterone is 1800 ng/dL.

QUESTION: What information is necessary to assess this patient's most recent serum testosterone level?

DISCUSSION: As men age, the testes decrease production of testosterone, the principal androgen in males. Whereas all men develop biochemical hypogonadism, when serum testosterone levels are below the normal range, only some men develop clinical symptoms that require medical intervention. This is similar to women who go through menopause. In the short term, hypogonadism is associated with decreased libido, erectile dysfunction, and mood changes. In the long-term, hypogonadism is associated with osteoporosis, weight gain, and decreased body muscle. For patients with confirmed hypogonadism-related decreased libido, erectile dysfunction, and mood changes, testosterone replacement therapy is effective in reducing these symptoms. The least expensive regimen is intramuscular injections of depot testosterone enanthate or testosterone cypionate, which are typically administered every three or four weeks. Serum testosterone levels should be obtained two or three months after the start of treatment, and the goal is to increase serum testosterone to the mid-normal physiologic range. Excessive doses of testosterone are associated with adverse effects including mood swings and polycythemia. Polycythemia is a direct result of the anabolic effects of testosterone and its stimulatory effect on erythropoiesis. In elderly patients, polycythemia may clog small capillaries, which may predispose to the development of a cerebrovascular accident, myocardial infarction, or priapism. Therefore, testosterone supplementation should be discontinued when the hematocrit exceeds 50%.

The serum testosterone level of 1800 ng/dL is high (normal range, 280–1100 ng/dL), and the usual target serum testosterone during treatment with a testosterone replacement regimen is 400–700 ng/dL. In assessing the increase in serum testosterone level, the following evidence strongly implicates testosterone enanthate as the cause of the elevated level: the serum testosterone increased after testosterone enanthate was started, and no other medications or diseases could be contributing to the increase in serum testosterone level. Parenteral testosterone enanthate is known to produce supraphysiologic serum testosterone levels at the time of peak absorption, which is 24 hours after a dose is administered. The key is to measure the level at the midpoint of the dosing interval to assess the adequacy of the dose that the patient is receiving.[11,12]

Before making an adjustment to the testosterone enanthate dose or the dosing interval, the clinician should ask for the timing of the blood draw relative to the timing of the most recent dose. If the blood sample was secured within 24 hours of the dose, then the serum testosterone level should be disregarded. Instead, a repeat serum testosterone level should be repeated at the midpoint of the dosing interval. If the serum testosterone level of 1800 ng/dL was obtained one week after the most recent dose, then this level indicates that the patient's dose is excessive. The dosing interval should be lengthened, probably to four weeks.

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of *Effects of Drugs on Clinical Laboratory Tests,* published in the journal *Clinical Chemistry* in 1972, 9000 such interactions were included.[13] In the second edition of the same publication, which was published in 1975, 16,000 such interactions were reported.[14] In 1997, this resource, which had been converted to an online searchable database, included over 135,000 interactions.[15] In 2014, this resource included 171,000 interactions.[16]

As for in vitro interferences, the number of drug–laboratory test interferences may be moderated over time because of newer, more specific laboratory test methodologies that minimize cross-reactions with drug metabolites or drug effects on reagents or laboratory reactions.[9,15] In addition, manufacturers of commonly used laboratory equipment systematically study the effects of drugs on assay methods.[17] Therefore, this information is often available to clinicians who confront problematic laboratory test results in patients. This increased awareness reduces the number of patients who are believed to have experienced newly reported drug–laboratory test interferences.

Suspecting a Drug Interference

A clinician should suspect a drug–laboratory test interference when an inconsistency appears among related test results or between test results and the clinical picture. Specifically, clinicians should become suspicious when the following occurs:

- Test results do not correlate with the patient's signs, symptoms, or medical history.
- Results of different tests—assessing the same organ anatomy or organ function, or the drug's pharmacologic effects—conflict with each other.
- Results from a series of the same test vary greatly over a short period of time and for no apparent reason.
- Serial test results are inconsistent.

No Correlation with Patient's Signs, Symptoms, or Medical History

As emphasized elsewhere in this book, when an isolated test result does not correlate with signs, symptoms, or medical history of the patient, the signs and symptoms should be considered more strongly than the test result. This rule is particularly true when the test result is used to confirm suspicions raised by the signs and symptoms in the first place or when the test result is used as a surrogate marker or indirect indicator of underlying pathology.

For example, serum creatinine is used in various formulae to approximate the glomerular filtration rate, which is used to assess the kidney's ability to make urine. However, actual urine output and measurement of urinary creatinine excretion is a more accurate method of assessing overall renal function. If a patient's serum creatinine has increased from a baseline of 1 mg/dL to 5 mg/dL over a three-day period, but the patient has had no change in urine output, urinary creatinine excretion, or serum electrolyte levels, then the serum creatinine level may be elevated because of a drug interference with the laboratory test. Similarly, if a patient has a total serum bilirubin of 6 mg/dL, but the patient is not jaundiced or does not have scleral icterus, then a drug interference with the laboratory test should be considered.

Conflicting Test Results

Occasionally, pharmacological or toxicological effects of a drug produce conflicting results of two tests that assess the same organ function. For example, a presurgical test screen shows a serum creatinine of 4.2 mg/dL in an otherwise healthy 20-year-old patient with a BUN of 8 mg/dL. Usually, if a patient had true renal impairment, BUN and serum creatinine would be elevated in tandem. Thus, in this patient, a drug interference with the laboratory test is suspected. Further investigation revealed that the patient received cefoxitin shortly before blood was drawn for the laboratory test. Cefoxitin can falsely elevate serum creatinine concentrations. Thus, the elevated serum creatinine is likely due to drug interference with the laboratory test and not to renal failure. To confirm that this is the case, cefoxitin should be discontinued and the serum creatinine repeated after that. If due to the drug, the elevated serum creatinine should return to the normal range.[18]

Varying Serial Test Results Over a Short Time Period

Typically, the results of a specific laboratory test should follow a trend in a patient. However, in the absence of a new onset of medical illness or worsening of existing disease, a sudden change in the laboratory test result trend should cause examination of a possible drug interference with a laboratory test. For example, prostate specific antigen (PSA) is a tumor marker for prostate cancer. It is produced by glandular epithelial cells of the prostate. The normal serum level is <4 ng/mL in a patient without prostate cancer, and the level is typically elevated in patients with prostate cancer. However, it is not specific for prostate cancer. Elevated PSA serum levels are also observed in patients with benign prostatic hyperplasia, prostatitis, or following instrumentation of the prostate. A 70-year-old male patient with stage T_3 (locally invasive) prostate cancer has a PSA of 20 ng/mL and has decided not to undergo treatment. Four serial PSA tests over the course of one year and done at three-month intervals show no change. Despite the absence of any changes on pelvic computerized axial tomography, bone scan, or chest x-ray, his PSA is 10 ng/mL at his most recent office visit. After a careful interview of the patient, the urologist discovers that the patient has been treated for androgenetic alopecia for the past six months with finasteride. The patient received the prescription from another physician for lower urinary tract voiding symptoms, and finasteride lowered the PSA level.[19]

Changing Serial Test Results That Are Inconsistent with Expected Results

Generally, repeated laboratory test results should show little change over time assuming that the status of the medical condition or treatment for the medical condition in an individual patient stays the same. However, when serial test results are inconsistent with expected results, a drug–laboratory test interference should be suspected. For example, leuprolide, a luteinizing hormone-releasing hormone (LHRH) agonist, is useful in the management of prostate cancer, which is an androgen dependent tumor. Persistent use of leuprolide causes downregulation of pituitary LHRH receptors, decreased secretion of luteinizing hormone, and decreased production of testicular androgens. A patient with prostate cancer, treated with leuprolide, should experience a sustained reduction in serum testosterone levels from normal (280–1100 ng/dL) to castration levels (<50 ng/dL) after two to three weeks. The serum testosterone level should remain below 50 ng/dL as long as the patient continues treatment with leuprolide, making sure that he makes visits to the clinic for repeated doses on schedule. However, one of the adverse effects of leuprolide is decreased libido and erectile dysfunction, which is a direct extension of the drug's testosterone-lowering effect. Such a patient may seek medical treatment of sexual dysfunction, and he may be inappropriately prescribed depot testosterone injections. Thus, in this case, depot testosterone injections will cause a change in serum testosterone levels in the wrong direction. If serum testosterone levels increase, this should be a signal that the patient has serial test results, which are inconsistent with expected results of leuprolide, and an investigation should be done as to the cause **(Minicase 1)**.[20]

MANAGING DRUG INTERFERENCES

When a drug is suspected to interfere with a laboratory test, the clinician should collect appropriate evidence to confirm the interaction. Important information includes the following:

1. Establishing a temporal relationship between the change in the laboratory test and drug use and ensuring that the change in the laboratory test occurred after the drug was started or after the drug dose was changed

2. Ruling out other drugs as causes of the laboratory test change
3. Ruling out concurrent diseases as causes of the laboratory test change
4. If possible, discontinuing the causative agent and repeating the test to see if dechallenge results in correction of the abnormal laboratory test
5. Choosing another laboratory test that will provide assessment of the same organ's function but is unlikely to be affected by the drug (the clinician can compare the new results against the original laboratory test result, and check for dissimilarity or similarity of results)[3]
6. Finding evidence in the medical literature that documents the suspected drug–laboratory test interference
7. Contacting the head of diagnostic labs who maintains or has access to computerized lists of drugs that interfere with laboratory tests (the person would also provide assistance in interpreting aberrant laboratory test results)[13]

For any particular patient case, it is often not possible to obtain information on all seven of the above items. The first four items are crucial in any suspected drug–laboratory test interference. With the availability of highly accessible, electronic databases—which can scour the literature quickly for drug–laboratory test interactions—and more electronic cross-talk between databases for clinical laboratory tests and those for medications, clinicians can easily find published information about drug interferences with laboratory tests; consult with clinical laboratory specialist, if necessary; and then take appropriate steps in managing the patient.[21,22]

LITERATURE RESOURCES

A systematic search of the medical literature is essential for providing the appropriate evidence to confirm the drug–laboratory test interaction. This search will ensure that a complete and comprehensive review—necessary in making an accurate diagnosis—has been done. When searching the literature, it is recommended to use the method originally described by Watanabe et al. and, subsequently, modified by C. F. Kirkwood.[23,24] Using this technique, the clinician would search tertiary, secondary, and then primary literature.

Tertiary literature includes reference texts and monograph databases, which provide appropriate foundational content and background material essential for understanding basic concepts and historical data relevant to the topic. *Secondary literature*, functioning as a gateway to primary literature, includes indexing and abstracting services (e.g., PubMed). *Primary literature* includes case reports, experimental studies, and other nonreview types of articles in journals about the topic. These represent the most current literature on the topic. By systematically scanning the literature in this order, the clinician can be sure to have identified and analyzed all relevant literature, which is crucial in developing appropriate conclusions for these types of situations.

Tertiary Literature

Tertiary literature, which contains useful information about drug–laboratory test interferences, includes the *Physicians' Desk Reference.* Each complete package insert included in this book contains a precautions section that includes information on drug–laboratory test interferences. However, it is important to note that the *Physicians' Desk Reference* does not include package inserts on all commercially available drugs, nor does it include complete package inserts for all of the products included in the text. Thus, additional resources will need to be checked, e.g., DailyMed by the National Institutes of Health (http://dailymed.nlm.nih.gov/dailymed/). Also, the drug monographs in the *AHFS Drug Information*, published by the American Society of Health-System Pharmacists, include a section on laboratory test interferences. Although the information provided is brief, it can be used as an initial screen. This resource is available electronically through MedicinesComplete (https://www.medicinescomplete.com/mc/).

One of the most comprehensive compilations of drug–laboratory test interactions is *Effects on Clinical Laboratory Tests: Drugs, Disease, Herbs, and Natural Products* by D. S. Young. Although originally published for many years as a special issue of the journal, *Clinical Chemistry*, it is available as a searchable online resource; access to it can be purchased from the American Association of Clinical Chemistry.[16] The content is searchable by the name of the laboratory test; specific drug, herb, or disease name; preanalytic variable; type of body fluid specimen; and specific laboratory test abnormality (i.e., whether an increase or decrease in laboratory result is expected). Search results include a short explanation of the drug–laboratory test interaction and a reference citation. If there are multiple publications about the drug–laboratory test interaction, each reference citation is listed separately with a short summary of the findings on the interaction. Details on the dosage of drug that produced the interference may or may not be provided. Hence, a clinician will need to obtain the original references and evaluate the data independently as a separate step. A complimentary one month free trial of this database is provided at http://clinfx.wiley.com/aaccweb/aacc/about **(Minicase 2)**.

A variety of other books about clinical laboratory tests are listed below. Some are comprehensive references while others are handbooks. All of them provide information about drug–laboratory test interferences. However, the reference texts are more complete than the handbooks. In addition, several comprehensive review articles include current information about drug–laboratory test interferences.

Micromedex Solutions, DynaMed Plus, and Lexicomp, are all online searchable databases. For every drug included in the system, information is available in a drug monograph format, and any information about drug–laboratory test interferences is included in the monograph. Although not always listed separately as a laboratory test interference, the information may be included in the adverse reaction, warning, or monitoring section of the monograph. In addition,

MINICASE 2

Trimethoprim–Sulfamethoxazole-Induced Hypoprothrombinemia

Sally S., a 65-year-old female patient, is started on trimethoprim–sulfamethoxazole 800/160 mg by mouth twice daily for an upper urinary tract infection due to *E. coli*. Antibiotic treatment will continue for 14 days. She has atrial fibrillation and is also taking digoxin 0.125 mg by mouth daily and warfarin 2.5 mg by mouth daily. She has been on warfarin for years and says that she is fully aware of all the DOs and DON'Ts of taking warfarin. Her international normalized ratio (INR) regularly and consistently is 2.5, which is therapeutic. She has no history of liver disease and appears healthy and well nourished. Prior to the start of the antibiotic, her serum sodium was 137 mEq/L, potassium 4 mEq/L, BUN 10 mg/dL, creatinine 1 mg/dL, and INR 2.5. After three days of antibiotics, a repeat INR is 5.2, and she complains of persistent nose bleeding, which stops for a few hours but then restarts again.

QUESTION: What do you think is causing the laboratory abnormality? How should this patient be managed?

DISCUSSION: Trimethoprim–sulfamethoxazole inhibits cytochrome 2C9, the principal hepatic enzyme that catabolizes warfarin, decreases vitamin K–producing bacteria in the gastrointestinal tract and displaces warfarin from its plasma protein-binding sites. A search of the medical literature documents multiple cases of enhanced warfarin effect when trimethoprim–sulfamethoxazole is taken concurrently.[26,27]

In this patient, the drug interaction occurred after trimethoprim–sulfamethoxazole was started. She is not taking any other medications that could cause the drug–laboratory test interaction and has no history of vitamin K deficiency or liver disease, which could be causing hypoprothrombinemia. To confirm that trimethoprim–sulfamethoxazole is causing the drug interaction, the physician could discontinue the drug and then see if her INR returns to the range of 2–3. However, because the trimethoprim–sulfamethoxazole–warfarin interaction is well known, a better approach might be to continue antibiotic treatment, hold warfarin until the INR has decreased to 2.5, and then resume warfarin at a reduced daily dose.

for some drugs, drug information questions and answers are included. To access relevant information, the clinician can search information using the name of the drug or the laboratory test. Often, the drug–laboratory test interference is assigned a severity rating (e.g., major or minor interference) as an indication of its clinical significance, and references to primary literature are available so that the reader can learn more. In addition, some local clinical laboratory websites (e.g., http://www.mayomedicallaboratories.com/interpretive-guide/index.html), may be convenient to access and use.[25]

SUMMARY

Although the number of drug–laboratory test interferences increases as the number of commercially available drugs increases, improved literature resources that compile information on this topic and improved assay methodologies have helped clinicians in dealing with suspected cases of this problem. Most drug–laboratory test interferences are due to in vivo effects of drugs; that is, the drug's pharmacological or toxic effects produce specific alterations in laboratory values. A drug–laboratory test interference should be suspected whenever a laboratory test result does not match the signs and symptoms in a patient, when the results of different tests that assess the same organ function or drug effect conflict with each other, or when serial laboratory test values vary greatly over a short period of time.

To determine if a drug is interfering with a drug–laboratory test, the clinician should, at a minimum, establish a temporal relationship between the change in the laboratory test and drug use; rule out other drugs and diseases as the cause; and discontinue the drug and repeat the laboratory test to see if dechallenge results in correction of the abnormal laboratory test. The literature should be checked to see if documentation of the drug–laboratory test interference can be found. The literature search should be systematic to ensure retrieval of the most comprehensive and current information. Therefore, the clinician should proceed from the tertiary to the secondary and then to the primary literature and use a variety of resources to arrive at a conclusion.

LEARNING POINTS

1. ***How would a clinician distinguish an in vivo from an in vitro drug interference with a laboratory test?***

   ANSWER: An in vivo interaction is characterized by an actual change in measured analyte concentration or activity prior to specimen collection and analysis. That is, the change in the measured analyte occurred in the patient. An in vitro interaction is characterized by a drug's physical presence in a body fluid or tissue specimen, which interferes with clinical laboratory testing during the analytical process. The interaction occurs outside the patient's body and after the specimen is collected from the patient.

2. ***What clinical situations may suggest a potential drug–laboratory test interaction?***

   ANSWER: A clinician should suspect a drug–laboratory test interaction when an inconsistency appears among related test results or between test results and the clinical presentation (i.e., the patient's signs and symptoms do not match). In addition, if test results vary greatly over a short period of time or if serial results of the same laboratory test are inconsistent, the clinician should evaluate the patient for this possibility.

3. ***What key information should a clinician collect to confirm that a drug is causing a laboratory test interaction?***

   ANSWER: The four key criteria in confirming the presence of a drug–laboratory test interaction include the following:

   1. Ensuring that the change in the laboratory test occurred after the drug was started
   2. Ruling out other drugs as causes of the laboratory test change
   3. Ruling out concurrent medical illness(es) as causes of the laboratory test change
   4. Stopping the drug and seeing if the laboratory test result returns to the predrug value

4. ***What type of literature resource should a clinician access first to review foundational information on a drug's adverse reaction profile and the likelihood that it could be causing an in vivo laboratory test abnormality?***

   ANSWER: Tertiary literature, which includes reference texts, review articles, and searchable databases, will provide good background information on medications. This information is helpful in understanding the primary literature on the topic.

RESOURCES

Books and Handbooks

AHFS drug information 2015. Bethesda, MD: American Society of Health-System Pharmacists; 2012.

Burtis CA, Bruns MD. Tietz fundamentals of clinical chemistry and molecular diagnostics. 7th ed. St. Louis: WB Saunders; 2015.

Chernecky CC, Berger BJ. Laboratory tests and diagnostic procedures. 6th ed. St. Louis: WB Saunders; 2012.

Dasgupta A, Hammett-Stabler CA, eds. Herbal supplements: efficacy, toxicity, interactions with western drugs and effects on clinical lab tests. Hoboken, NJ: Wiley; 2011.

Laposata M, ed. Laboratory medicine: the diagnosis of disease in the clinical laboratory. New York: McGraw Hill Medical; 2014.

McPherson RA, Pincus MR, eds. Henry's clinical diagnosis and management by laboratory methods. 22nd ed. Philadelphia: Elsevier WB Saunders; 2011.

PDR Network. Physician's desk reference 2015. 69th ed. Montvale, NJ: PDR Network; 2015.

Williamson MA, Snyder LM. Wallach's interpretation of diagnostic tests: pathways to arriving at a clinical diagnosis. 10th ed. Philadelphia: Wolters Kluwer Lippincott Williams & Wilkins; 2015.

Young DS. Effects of preanalytic variables on clinical laboratory tests. 3rd ed. Washington, DC: American Association for Clinical Chemistry; 2007.

Young DS. Effects on clinical laboratory tests: drugs, disease, herbs, and natural products. 5th ed. Washington, DC: American Association for Clinical Chemistry; 2014.

Review Articles

- DasGupta A, Bernard DW. Herbal remedies: effects on clinical laboratory tests. *Arch Pathol Lab Med*. 2006; 130:521-8.

  This review summarizes literature from 1980–2005 on herbal drug interactions with laboratory tests. Mechanisms include (1) herbal agent-induced in vivo toxic effects; (2) direct assay interference by the herbal agent; or (3) contaminant in the herbal agent produces in vivo or in vitro effects that produce changes in laboratory test results. The effect of Chan su on digoxin blood levels and St. John's wort on blood levels of cyclosporine, digoxin, theophylline, and protease inhibitors are just some of the herbal agent–laboratory test interactions discussed. This is a follow-up to the author's first article on the topic, which was published in the *American Journal of Clinical Pathology* in 2003.
- Kroll MH, Elin RJ. Interference with clinical laboratory analyses. *Clin Chem*. 1994; 40:1996-2005.

  This is an excellent overview of drug–laboratory test interactions. The article describes how drugs, metabolites, and additives (e.g., heparin, ethylenediamine tetra-acetic acid) can produce significant interactions and discrepancies during in vitro analytic procedures. It also provides a summary of useful references (although outdated) on the topic. In addition, a suggested approach to drug–laboratory test interactions is described.
- Sher PP. Drug interferences with clinical laboratory tests. *Drugs*. 1982; 24:24-63.

  This useful reference provides many tables of drugs known to interfere with various laboratory tests. The data are arranged by laboratory test. For many common laboratory tests, summary tables of drugs known to interfere with the particular laboratory tests are provided. Also, mechanisms for the in vivo and in vitro interactions are described. Although this reference is dated and is not useful for newer drugs, it is an excellent resource for older drugs.
- Sonntag O, Scholer A. Drug interference in clinical chemistry: recommendation of drugs and their concentrations to be used in drug interference studies. *Ann Clin Biochem*. 2001; 38:376-85.

  In 1995, 18 clinical laboratory test experts identified 24 commonly used drugs known to interfere with laboratory tests. Usual therapeutic and toxic drug concentrations were identified. Both concentrations of each drug were added in vitro to blood and urine specimens and then various laboratory tests were run on the specimens. Laboratory testing was duplicated in three different laboratories. This review article summarizes drug–laboratory test interactions for over 70 different laboratory tests.

Secondary and Primary Literature

For secondary literature, the main indexing or abstracting service that should be used is PubMed. This allows the clinician to check the literature from thousands of biomedical journals from 1950 to the present. Due to improvements in search capabilities, clinicians can search using text words (i.e., words as they might appear in the title or abstract of a journal article). The database will automatically convert that text word to official medical subject headings or accepted indexing terms. As a result, search output is optimized despite the lack of proficiency or experience of the searcher. If the search represents a combination of concepts, the concepts can be appropriately linked using Boolean logical operators (e.g., or, and, and not). In addition, the database provides links enabling clinicians to locate related articles or order articles online, which enhance search capabilities and convenience in obtaining relevant primary literature articles.

This chapter does not allow a complete tutorial on search strategy development and conducting PubMed searches. However, the reader is encouraged to develop expertise in this area so that he or she can identify current, relevant literature efficiently. A wide variety of tutorials and webcasts are available free of charge (https://www.nlm.nih.gov/bsd/disted/pubmed.html).

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4

POINT-OF-CARE TESTING

Paul O. Gubbins and Heather Lyons-Burney

OBJECTIVES

After completing this chapter, the reader should be able to

- Describe clinical opportunities for point-of-care testing in a community pharmacy or ambulatory care setting for both population and patient-specific applications
- Differentiate between the values of sensitivity, specificity, accuracy, precision, positive predictive value, and negative predictive value when interpreting the results of a point-of-care test
- Discuss the limitations for CLIA-waived point-of-care testing
- Identify potential resources for CLIA-waived point-of-care testing options
- Identify potential resources for maintaining good laboratory practices

The pharmacy profession's role in the healthcare system is continually evolving. Over time, the profession has shifted from being product-focused to delivering patient-oriented pharmaceutical care. Today, pharmacists are on the frontline of providing patient-centered care and wellness. Moreover, their role in delivering care has tremendous potential to expand as healthcare delivery becomes more patient-centered and team-based. Pharmacists are highly trained, accessible healthcare professionals who are second only to registered nurses in terms of the number of practicing professionals. They are also underutilized in the U.S. healthcare delivery system.[1] However, in collaboration with physicians and other healthcare professionals, the role and ability of pharmacists can be maximized to deliver quality patient-centered care and improve public health.[1]

Clinical Laboratory Improvement Amendments of 1988 (CLIA)-waived point-of-care (POC) testing (POCT) represents one means by which pharmacists can improve the delivery of healthcare.[2] Working in collaboration with other providers and public health officials, pharmacists can leverage their knowledge and accessibility to offer CLIA-waived POCT services to manage chronic illnesses, improve access to healthcare services, rapidly initiate appropriate therapy, and screen for diseases of public health significance.[2] Chapter 1 defines POCT and differentiates it from home testing, and provides an overview of the advantages and disadvantages of these testing paradigms. The objective of this chapter is to identify opportunities to perform CLIA-waived POCT in ambulatory care clinics and community pharmacies. This chapter will focus on POC tests and POCT by expanding on the overview of common CLIA-waived POC tests provided in Chapter 1; in addition, this chapter will discuss available tests, review their performance measures and practical limitations, discuss their use in current practice, and identify potential future applications for their use in practice.

REGULATORY OVERSIGHT OF CLIA-WAIVED POC TESTS AND TESTING

The U.S. Food and Drug Administration (FDA) and the Centers for Medicare & Medicaid Services (CMS) are the federal agencies charged with oversight of CLIA. The FDA is responsible for classifying tests based on their level of complexity and potential for risk to public health. During the premarket approval process, the FDA classifies tests based on complexity (high, moderate, or waived) using criteria in the CLIA regulations. Waived tests are low-complexity methods that are simple to use, and their risk of producing erroneous results is negligible or poses no reasonable risk of harm to the patient if performed incorrectly.[3]

CMS regulates facilities that conduct laboratory testing on human specimens for health assessment, diagnosis, prevention, or treatment of disease, including all POC tests. Waived laboratories, such as community pharmacies or ambulatory care clinics, can only perform waived tests and are not subject to regular inspections, personnel requirements, or proficiency testing. To perform such tests, these sites must obtain a CLIA Certificate of Waiver from CMS, pay applicable fees biannually, and follow the manufacturers' test instructions. In most states the process is similar; however, CMS has exempted New York and Washington from CLIA so some of the processes and regulations to perform CLIA-waived tests are different. More information on how to apply for a CLIA Certificate of Waiver can be found at the CMS website (https://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA/How_to_Apply_for_a_CLIA_Certificate_International_Laboratories.html).

COMMON CLIA-WAIVED POC TESTS

Resources

Because of recent technological advances, the market for CLIA-waived POC tests is continually and rapidly growing and changing. Therefore, it is important for a pharmacist in a community pharmacy or ambulatory care clinic that has obtained a CLIA waiver to be aware of the most current information on the available tests. In addition to FDA and CMS regulatory oversight, the Centers for Disease Control and Prevention (CDC) provide support for the CLIA program and offer an additional resource for information on CLIA-waived testing. Useful and current information on CLIA-waived tests is available on the websites of all three agencies.

FDA Website

Perhaps the most up-to-date, comprehensive, and readily searchable resource is the FDA's list of analytes that are used in waived laboratory test systems, which is located at http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfClia/analyteswaived.cfm. This resource is an alphabetically organized list of analytes hyperlinked to test and regulatory information. When a specific analyte (e.g., cholesterol) is selected, a list of waived test systems that measure the analyte appears (each with hyperlinks to regulatory information and documentation). The advantage of this resource is that it is updated frequently. However, it contains more regulatory information than most clinicians in a clinical practice site need.

CMS Website and Guidance Document

A document listing tests that have been granted a waived status under CLIA may be downloaded from the CMS website at https://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA/Downloads/waivetbl.pdf. This resource is a list of tests organized by Current Procedural Terminology (CPT) code. Although this resource does not contain as much information as that found on the FDA website, it contains the basic information (e.g., CPT code, test name, and manufacturer) that clinicians may find useful in a clinical setting in an easy-to-read tabular format.

CDC Website

The CDC's website (https://wwwn.cdc.gov/clia/default.aspx) is a good resource to search for professional information and educational resources regarding the analytical and technical aspects of a test for a given analyte. In addition, the CDC's website is specific for CLIA-waived POC tests (https://www.cdc.gov/clia/Resources/WaivedTests/) and contains links to documents that outline good laboratory practices for sites performing waived tests and a booklet that details the practical considerations for performing CLIA-waived POC tests or developing CLIA-waived POCT services.[4,5]

Specimens Used in CLIA-Waived POC Tests for Chronic Disease State Management

Waived tests are approved for use only with unprocessed specimens that require no manipulation (e.g., centrifugation, precipitation, dilution, and extraction). Specimens, such as serum or plasma, require manipulation during sample preparation or training in their handling such that they are not suitable for use in CLIA-waived POC tests.[6] Clinicians should be aware that some test systems provide instructions for processed and unprocessed specimen types, but waived use is intended only for the testing of unprocessed specimens. In addition, depending on the type of specimen the test analyzes, not all CLIA-waived POC tests used for disease state management are suitable or feasible for use in a community pharmacy or ambulatory care clinic. The most commonly obtainable specimen types for POCT in disease state management are urine and whole blood.

Urine

The urine dipstick and tablet reagent urinalysis are common CLIA-waived POC tests found in many ambulatory care settings. The urinalysis test provides pharmacists with values for bilirubin, glucose, hemoglobin (Hgb), ketone, leukocytes, nitrite, pH, protein, and specific gravity. This test can be used to detect an acute urinary tract infection. The pharmacist involved in a smoking cessation program might use a nicotine detection test. This test detects nicotine and its metabolites in urine and could indicate the smoking status of an individual as a low or high nicotine consumer. Urine tests can also be used for toxicological screenings and for drug metabolism phenotyping. However, these uses are beyond the scope of this chapter.

Whole Blood

Pharmacists obtain whole blood samples through a finger stick method for a variety of CLIA-waived POC tests. Blood conservation is one advantage of POC tests; as such, tests analyze whole blood analytes using volumes typically measured in drops of blood rather than milliliters. Each testing device may require varying amounts of a blood sample for a given analyte, making it critical for pharmacists to follow the manufacturer's guidelines for blood sample collection as required by CLIA-waived testing regulations. The minimal amount of blood required by POC tests may also reduce the chance of errors that can occur when using larger volumes. As described in Chapter 1, a quick turnaround time (TAT) is also a major advantage to POCT. The TAT is the time interval from sample collection to test performance, and it is a critical step in ensuring test accuracy for some CLIA-waived POC tests. Ambulatory care clinics and community pharmacies are often very busy; thus, pharmacists performing CLIA-waived POCT services in these settings must be cognizant of proper sample collection technique and timing information. This information is also found in the manufacturer's guidelines.

A wide variety of CLIA-waived POCT devices are able to measure blood chemistry in an ambulatory care clinic or community pharmacy. A blood chemistry analyzer uses a whole blood sample and can measure analytes including ionized calcium, carbon dioxide, chloride, creatinine, glucose, potassium, sodium, urea nitrogen, and hematocrit (Hct). In addition, some CLIA-waived blood chemistry analyzers are capable of measuring other analytes including alanine amino transferase, aspartate amino transferase, albumin, total bilirubin, alkaline phosphatase, and total protein. Blood chemistry analyzers with these analytical capabilities can be used by a pharmacist practicing under a collaborative practice agreement (CPA) or collaborative drug therapy management (CDTM) provisions in state regulations or statutes to determine liver and renal function values and assist with any appropriate medication dosing adjustments for chronic conditions, such as diabetes or cardiovascular disease.

Measurement of the percent concentration of glycated hemoglobin (HgbA1C) in blood is a useful CLIA-waived POC test for the monitoring of patients with diabetes during routine follow-up visits. In addition, CLIA-waived POC tests that measure total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides in whole blood can be used by the pharmacist in the management of patients with chronic diseases such as diabetes, dyslipidemia, and cardiovascular disease. Pharmacists are often involved in CPAs for managing patients requiring medication dosage adjustments based on CLIA-waived test results. The prothrombin time/international normalized ratio monitoring systems and the thyroid- stimulating hormone whole blood tests are common examples of such tests. A CLIA-waived POCT device for Hgb and Hct can allow the pharmacist to monitor therapy for anemia or other blood conditions. Immunoassay tests for detection of antibodies specific to *Helicobacter pylori* in whole blood can be utilized by pharmacists to determine appropriate medication therapy for peptic ulcer disease or monitor *H. pylori* eradication therapy. Direct pharmacist involvement in medication therapy through use of CLIA-waived testing can assist patients in reaching their chronic disease management goals, as well as building strong patient and pharmacist relationships.

Specimens Used for CLIA-Waived POC Tests for Infectious Diseases Screening and Management

Currently, there are CLIA-waived tests for 16 infectious diseases analytes, but not all of these tests have POC applications or are suitable for testing in either the ambulatory care clinic or community pharmacy settings. Of the 16 analytes, the most common analytes in these settings are group A streptococci (GAS), human immunodeficiency virus (HIV), seasonal influenza A and B, and hepatitis C virus (HCV). The most common obtainable specimen types for POCT for screening or management of infectious diseases are oral mucosal transudate, whole blood, and swabbed secretions from nose, nasopharynx, and oropharynx.

Secretions from the Nose, Nasopharynx, and Oropharynx

Two infectious disease analytes, GAS and influenza A and B, cause acute respiratory illnesses. The upper respiratory tract—the nasopharynx, oropharynx, and laryngopharynx—is easily accessible to airborne microorganisms and is colonized throughout life by commensal organisms and potential bacterial pathogens including GAS, but not by viruses such as influenza A and B. For the most part, the upper respiratory tract with its lymphoid tissue and normal flora acts as a barrier to pathogens reaching the lower respiratory tract. Acute respiratory illnesses can occur when this barrier is overcome by the proliferation of colonizing bacteria from the different areas of the upper respiratory tract. Collecting lower respiratory tract specimens such as sputum for diagnostic purposes often requires invasive procedures and for this reason, CLIA-waived POC tests for GAS and influenza A and B rely on obtaining secretions from the more readily accessible upper respiratory tract. With minimal training, pharmacists can perform nasopharyngeal, nasal or oropharyngeal swab to collect specimens that would be suitable for the available CLIA-waived POC tests. Such samples can also be collected by nasopharyngeal aspiration, but this method requires a sterile suction catheter, suction apparatus, and transport media, whereas collecting the specimen via a swab requires only a sterile swab and transport media. Although nasal aspiration is very sensitive, it is technically difficult and likely not practical for a pharmacist to perform in an ambulatory care clinic or community pharmacy setting.[7,8]

Oral Mucosal Transudate

To date, HIV-1 and HIV-2 are the only infectious disease analytes measured from these specimens for CLIA-waived tests. In 2004, the FDA approved a rapid HIV-antibody-based, CLIA-waived POC test—which it had initially approved for finger stick, whole blood, and plasma specimens—for use with specimens of oral mucosal transudate. In 2012, the FDA approved an identical version of the test for sale directly to consumers for in home use, which cannot be used in ambulatory care clinics or community pharmacies. Oral mucosal transudate is more acceptable to patients because of its noninvasive and pain-free specimen collection and its rapid TAT. Moreover, the test enabled the expansion of testing efforts from inpatient facilities to outpatient, community health, and nonclinical outreach testing sites, thereby increasing the availability of HIV testing and allowing more people to get tested and learn their results in a timely manner. Thus, the POC test aligns with the 2006 CDC recommendation for routine opt-out screening for individuals aged 13–64 years to help reduce the spread of the virus.[9] With minimal training, pharmacists can swab a person's oral cavity to obtain oral mucosal transudate and perform the professional version of the CLIA-waived test. In general, POC tests for HIV-1 or HIV-2 using oral mucosal transudate specimens are highly accurate, are less technically demanding than methods using blood, and minimize the concern for biohazard disposal. The POC test is not diagnostic, and in the acute phase of infection before seroconversion when HIV antibody titers are low, oral testing has low sensitivity and may miss more acute HIV infections than CLIA-waived tests that use whole blood specimens.

Whole Blood

HIV-1 and HIV-2, and HCV are the only infectious disease analytes measured from whole blood for CLIA-waived POC tests. Like POC tests for chronic disease state management, many pharmacists are comfortable with obtaining whole blood samples through a finger stick method for a variety of CLIA-waived POC tests. Unlike the CLIA-waived oral tests for HIV-1 and HIV-2, the CLIA-waived whole blood tests for HIV-1 and HIV-2 require more equipment (e.g., lancets) and biohazard waste precautions (e.g., sharps containers and gloves). Although a blood sample for HIV-1, HIV-2, and HCV CLIA-waived tests that use whole blood can be obtained by venipuncture, it is easier to obtain the sample via finger stick for POCT purposes in ambulatory care clinics or community pharmacy settings.

Uses of CLIA-Waived POC Tests for Chronic Disease State Management and Infectious Diseases

CLIA-waived POC tests for chronic disease state management are used both in the screening for risk of certain disease states (e.g., diabetes and dyslipidemia) and in their long-term management. Most CLIA-waived POC tests for infectious diseases are used either to screen for infections of public health interest (e.g., HIV and HCV) or to aid in the diagnosis and management of an acute infection in an individual patient (e.g., GAS). The CLIA-waived POC tests for influenza A and B can be used for either application.

Use in Screening Efforts

Pharmacists can perform screening for diabetes and dyslipidemias as part of large community or corporate health and wellness efforts. Individuals identified as being at risk can then be offered counseling on nonpharmacologic lifestyle changes (e.g., exercise, dietary adjustments, and smoking cessation) or referred to their provider for additional follow-up. It is important that pharmacists reference national disease state guidelines when discussing results and determining the appropriate time to refer patients to their primary care provider.

Chronic Disease State Management

Training pharmacists on the application and proper use of CLIA-waived POC tests for the management of chronic diseases could produce significant benefits to community health. An estimated 21 million Americans have been diagnosed with diabetes mellitus with an additional 8.1 million remaining undiagnosed.[10] Approximately 70 million (33%) U.S. adults 20 years of age or older have hypertension, of which only 52% of those diagnosed are controlled to target levels.[11] In 2010, coronary heart disease is estimated to have caused one of every four deaths in the United States.[12] Hyperlipidemia, total serum cholesterol >240 mg/dL, affects an estimated 31.9 million adults.[13] Chronic disease management for diabetes, hypertension, cardiovascular disease, and hyperlipidemia are areas in which pharmacists have demonstrated positive impact on outcomes.[1] These conditions require prescription medication therapies in addition to nonpharmacological lifestyle changes. In addition, clinical management guidelines for these chronic disease and the medications used in their treatment recommend regular monitoring of laboratory values to determine appropriate drug dosing. Such monitoring can often be done using CLIA-waived POC tests.

Uses in Screening for Infections of Public Health Interest

The burden of disease for the available infectious disease POC tests is substantial. Estimates suggest that more that 1.2 million individuals in the United States are living with HIV infection, of which 168,300, or one in eight (13%), are unaware of their infection.[14] Individuals who are unaware of their HIV infection status contribute markedly to the annual rate of newly infected individuals. In 2006, the CDC recommended routine opt-out screening for individuals aged 13–64 years; by 2012, approximately 45% of surveyed Americans reported ever being tested.[9,15] Likewise, it is estimated 2.7 to 3.2 million people in the United States are chronically infected with HCV, and most infected individuals are unaware of their infection because they are asymptomatic.[16-19] Recent CDC estimates suggest there were nearly 30,000 new infections in 2013.[20] Lastly, annually up to 20% of Americans are infected by influenza. Seasonal influenza infection and its complications results in more than 200,000 hospitalizations a year, particularly among the very young and the elderly.[21]

Given the accessibility of pharmacies, training pharmacists on the application and proper use of CLIA-waived POC tests for the screening of HIV and HCV could improve testing rates; increase the linkage to medical treatment (e.g., facilitate medicine distribution and access to medication assistance programs); expand access to appropriate counseling services, community resources, and care; aid in prevention efforts (e.g., needle-exchange programs); and enhance collection of prevalence and surveillance data to help resources reach targeted at-risk populations quicker. In short, pharmacist-directed CLIA-waived POCT efforts could potentially have a significant impact on public health efforts to increase the number of people getting tested, especially those who engage in high-risk behaviors. Moreover, using CLIA-waived POC tests to screen for influenza represents a collaborative opportunity for pharmacy and local health departments to improve data sharing to inform disease surveillance efforts. In some cases, such efforts can be combined with technological solutions to improve vaccine and antiviral distribution and perhaps even curtail inappropriate antibacterial use.

Uses in Diagnosis and Management of an Acute Infection in an Individual Patient

Pharmacists can also use certain POC tests for infectious diseases to assist in the acute management of individual patients. For example, GAS causes 5–10% of adult acute pharyngitis and 15–30% of acute pharyngitis among children.[22] Epidemiologically GAS pharyngitis occurs primarily in the winter and early spring and afflicts individuals in a narrow age range. Although the infection has a well-recognized presentation and rarely requires confirmation by culture, clinically its symptoms are often indistinguishable from viral etiologies. Rapid diagnostic CLIA-waived POC tests can help ensure that appropriate antibiotic therapy is initiated, if warranted, which could hasten infection resolution by one to two days. Influenza is another infection that occurs seasonally, and its symptoms are often indistinguishable from bacterial etiologies. Moreover, prompt initiation of appropriate cost-effective antiviral therapy is key to hastening resolution of the infection and limiting its severity.

POC TEST PERFORMANCE CHARACTERISTICS

Use of Performance Characteristics When Choosing and Performing CLIA-Waived POC Tests

As discussed in Chapter 1, tests can be qualitative, quantitative, or semiquantitative. In general, although some CLIA-waived POC tests for chronic disease management that are applicable to the ambulatory care clinic or community pharmacy settings may be semiquantitive, most are either quantitative or qualitative. In contrast, currently all CLIA-waived POC tests for infectious diseases that are applicable to the ambulatory care clinic or community pharmacy settings are qualitative. Regardless of whether a test is qualitative, quantitative, or semiquantitative, as discussed in Chapter 1, CLIA-waived POC tests can be described in terms of several performance characteristics including accuracy, precision, reliability sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).

Accuracy and Bias

The terms *accuracy* and *bias* are often used synonymously, but the former is a qualitative term, whereas the latter is a quantitative term. For a quantitative CLIA-waived POC test, bias characterizes how close the mean measurement of the test is to the true value of the analyte in the specimen of interest. Qualitative tests measure only the presence or absence of the analyte, and because of inherent error they can produce positive or negative results, which are either true or false. Thus, for qualitative CLIA-waived POC tests, accuracy measures how well the test identifies or excludes the presence of the analyte. Accuracy characterizes the number of true results, both positive and negative, relative to the total number of samples tested; thus, it is the percentage of true results.

$$\textbf{\textit{Accuracy}} = \frac{\textit{Number of true positives} + \textit{Number of true negatives}}{\textit{Number of true positives} + \textit{False positives} + \textit{True negatives} + \textit{False negatives}}$$

For example, if specimen samples containing a known analyte and specimen samples without the analyte are repeatedly measured by a CLIA-waived POC test, and the percent yield of true results is high, the test would be considered highly accurate.

Precision

For a CLIA-waived POC test, *precision* characterizes test reproducibility or the degree to which the test performed under constant conditions will produce the same measurement each time. For a quantitative test, precision is numerically characterized by values such as standard deviation, relative standard deviation (coefficient of variance), or standard error of the mean, (which are all measures of imprecision). A CLIA-waived POC quantitative test with high precision will regularly produce results that are in close agreement. For qualitative tests, precision or PPV is the proportion of true positive results relative to all (i.e., true and false) the positive results. A CLIA-waived POC quantitative test with high precision will regularly produce results that are truly positive for the analyte of interest.

Sensitivity

CLIA-waived POC tests for chronic disease management that are practical for the ambulatory care clinic or community pharmacy setting are used to evaluate the need for therapy adjustments at a certain treatment threshold, or in the case of infectious diseases, confirm the presence of an infectious disease and aid in its diagnosis. If a CLIA-waived POC test for an infectious disease was perfect, it would produce a positive result in all patients with the infection and a negative result in all patients who are not infected. Unfortunately, like laboratory-based tests for infectious diseases, no CLIA-waived POC test for an infectious disease is perfect.[23] Thus, when the tests are evaluated via comparison to a referenced standard, the terms *sensitivity* and *specificity* are used to characterize the test's ability to differentiate between truly positive and truly negative results. However, at times manufacturers evaluate their test via comparison to a nonreference standard, in which case the estimates are called *positive percent agreement* and *negative percent agreement*, rather than sensitivity and specificity, respectively.[24]

The *sensitivity* of a quantitative test is dependent on a predefined cutoff or threshold value, so that a diagnosis or a therapeutic management decision can be made. Because CLIA-waived POC tests for infectious diseases are qualitative, sensitivity assesses the test's ability to detect the infection of interest in a patient who is truly infected (i.e., a true positive). No test is perfect (i.e., 100% sensitive); thus, sensitivity is the proportion of true positives the test correctly detects among all those who are infected (i.e., correctly identified positive plus the incorrectly undetected).

$$\textbf{\textit{Sensitivity}} = \frac{\textit{Number of true positives}}{\textit{Number of true positives} + \textit{Number of false negatives}}$$

The calculation for positive percent agreement is identical[24]. A CLIA-waived POC test for infectious diseases that is 80%

sensitive will positively detect 80% of the people who have the infection, but it will not detect the other 20% who are also infected (i.e., false negatives). A high test sensitivity lowers the chance of a false-negative result and vice-versa. Thus, highly sensitive tests are preferred for a CLIA-waived POC test for an infectious disease that carries a poor prognosis (i.e., a false negative cannot be tolerated).

Specificity

In contrast to sensitivity, *specificity* of a qualitative test for infectious disease assesses the test's ability to not detect the infection of interest in a patient who is not infected (i.e., a true negative). Specificity is the proportion of true negatives the test correctly identifies among all those who are not infected (i.e., correctly identified negative plus the incorrectly detected).

$$\boldsymbol{Specificity} = \frac{Number\ of\ true\ negatives}{Number\ of\ true\ negatives + Number\ of\ \ false\ positives}$$

The calculation for negative percent agreement is identical.[24] Thus a CLIA-waived POC test for infectious diseases that is 90% specific will incorrectly detect the infection in 10% of the people who are not infected (i.e., false positives). A highly specific test lowers the chance of a false-positive result and vice versa. For a CLIA-waived POC test for an infectious disease, a highly specific test could be used to confirm a diagnosis because it would rarely be positive in the absence of the infection. Like sensitivity, the specificity of a quantitative test is dependent on a cutoff value, which is also known as the *limit of detection*.

Positive Predictive Value

The *positive predictive value* (PPV) allows clinicians to apply a test's performance characteristics to their own clinical settings. For a CLIA-waived POC test for infectious diseases, the PPV addresses the likelihood that a given patient has the infection of interest when the test result is positive. The PPV is the proportion of true positives relative to all positive (true or false) results.

$$\boldsymbol{PPV} = \frac{Number\ of\ true\ positives}{Number\ of\ true\ positives + Number\ of\ \ false\ positives}$$

Negative Predictive Value

The *negative predictive value* (NPV) also allows clinicians to apply a test's performance characteristics to their own clinical settings. For a CLIA-waived POC test for infectious diseases, the NPV addresses the likelihood that a given patient does not have the infection of interest when the test result is negative. The NPV is the proportion of true negatives relative to all negative (true or false) results.

$$\boldsymbol{NPV} = \frac{Number\ of\ true\ negatives}{Number\ of\ true\ negatives + Number\ of\ \ false\ negatives}$$

Unlike sensitivity and specificity, PPVs and NPVs are not inherent to the test, but they are dependent on the population being tested and are influenced by the prevalence of the disease. This means that there may be strategies to improve these values and thereby the usefulness of a test, which will be discussed later in the chapter.

Interferences

Interferences (or analytic specificities) are a performance characteristic that is not statistically based. Rather, interferences are medical conditions, medications, or other substances that might influence test results positively or negatively. For quantitative tests, interference can obscure the limit of detection, whereas with qualitative tests it could cause falsely positive or falsely negative results. Interferences with CLIA-waived POC tests can occur and often involve microbial cross-reactivity, microbial interference, or other interfering substances such as chemicals or certain foods. Information on interferences is included in the manufacturer information included with the tests, which personnel performing the test must read to ensure they have the most up-to-date information.

Performance Characteristics of Specific Tests

Like any test, CLIA-waived POC tests have inherent error associated with their use. Thus, clinicians should be aware of the variability of POC tests when interpreting their results. A given test's performance measures will be included in the manufacturer's test literature. In addition, such information may be gleaned from the literature or the manufacturers' websites.

HgbA1C

Prior to the early-1990s, measurement of HgbA1C was not standardized so it was used only as a surrogate marker of glycemic control. However, in the early 1990s the landmark Diabetes Control and Complications Trial (DCCT) established the correlation between HgbA1C measurement and the risk of developing diabetic complications.[25-27] In doing so, target values were created to lower patients' risk of a poor outcome.[25,26] Rigorous standardization was needed because in the DCCT a very small difference, such as 2%, in mean HgbA1C values between treatment groups translated into a significant decrease in the risk for a variety of diabetic complications.[25] The National Glycohemoglobin Standardization Program (NGSP) was initiated to standardize HgbA1C test results so that laboratory results are comparable to those in the DCCT.[27] As part of the NGSP, a network of laboratories works with test manufacturers to standardize their methods.[27] Over the years, the rigorous standardization efforts have facilitated the monitoring of HgbA1C with rapid reporting of results, which has demonstrated improved glycemic control in type 1 and insulin-treated type 2 patients with diabetes.[28,29]

The American Diabetes Association recommends that the HgbA1C methods used be NGSP certified.[30] Although the same quality standards apply for laboratory and POC HgbA1C systems, a concern regarding the lack of required proficiency testing for end users performing CLIA-waived POC HgbA1C tests exists.[25,26] Few data characterize how these methods actually perform when conducted in the setting of a CLIA-waived laboratory; thus, their real analytical performance is not known.[25,26] Nonetheless, data indicate that POC methods for HgbA1C, as a whole, perform no worse than many laboratory-based HgbA1C methods.[25] Moreover, in a series of studies, investigators have demonstrated that the analytical performance of POC instruments for HgbA1C have improved considerably in the last half decade.[31,32]

Measurement of HgbA1C via POC tests are based on structural differences among the various types of Hgb molecules, using either affinity separation or more specific immunoassays.[26] Affinity separation methods use a boronate matrix to measure "total" glycation and distinguish glycated Hgb from nonglycated Hgb.[26] Based on affinity separation, the analyzers of the CLIA-waived POC tests measure the percentage of HgbA1C. An advantage to affinity separation methods is a lack of interference by nearly all Hgb variants or derivatives.[26] Immunoassay methods use specific antibodies directed toward the first several amino acids and the glucose molecule of the N-terminal of the β-chain of the Hgb molecule.[26] These methods measure total Hgb through methods including turbidimetric measurement and latex agglutination inhibition.[26] Although most immunoassays do not interfere with common Hgb variants (e.g., HbAS, HbAC, HbAD, and HbAE), they are subject to interference with rare Hgb variants resulting from amino acid substitutions.[26]

In evaluating the performance characteristics of CLIA-waived POC, HgbA1C tests bias, and measures of imprecision are important.[25] As the measurement of HgbA1C can result in therapy adjustments, consistency between POCT devices and methods is an important component of a laboratory's policies and procedures. Therefore, using certified analyzers and tests will help ensure accurate and standardized results.

Cholesterol

Coronary heart disease (CHD) has a profound effect on the U.S. population as the leading cause of death among men and women affecting all racial and ethnic groups.[33] Historically, the National Cholesterol Education Program's goal to reduce morbidity and mortality because CHD relied on accurate and precise measurement of the lipid profile. In response to the need to improve cholesterol measurement, the CDC created the Cholesterol Reference Method Laboratory Network to ensure that manufacturers of diagnostic products in meeting the criteria of the National Reference System for Cholesterol (NRS/CHOL).[34] The NRS/CHOL determines the methods and materials for cholesterol testing that are utilized by research laboratories, which may determine the American College of Cardiology and American Heart Association prevention guidelines for healthcare professionals on the treatment and management of blood cholesterol to reduce atherosclerotic cardiovascular risk.

Several of the CLIA-waived tests have demonstrated acceptable ranges of sensitivity and specificity when compared to test methods from the Clinical and Laboratory Standards Institute (CLSI).[35] Many waived tests and devices are available for the testing of cholesterol that report results ranging from only total cholesterol to an entire standard lipid panel (e.g., total cholesterol, HDL, LDL, and triglycerides). These tests and devices typically use finger stick capillary or venous blood samples and produce results within a few minutes. One such CLIA-waived POC test, the Alere Cholestech LDX Analyzer, combines enzymatic methodology and solid-phase technology to determine analyte values. Another CLIA-waived test system, the Cardio-Chek PA Analyzer, is a handheld, portable device that uses reflectance photometry to produce results in one to two minutes. The procedure manuals for each test provide guidance on proper specimen collection, handling, and quality control measures. Variants, including day-to-day or seasonal variations, that can contribute to the accuracy of a cholesterol level include age; gender; diet and alcohol; exercise; medications; fasting; trauma or acute infection; or pregnancy.[36,37] The accessibility of the ambulatory care or community pharmacist in reaching large numbers of adults at risk for CHD with appropriate cholesterol testing, may benefit the prevention and treatment efforts in a community.

Hemoglobin

Anemia is typically caused by impaired production or increased destruction of red blood cells, blood loss, or fluid overload. Several CLIA-waived POC tests for *hemoglobin* (Hgb) using capillary blood samples have been utilized in ambulatory care settings to assist with the diagnosis of anemia or its associated morbidities. The CLIA-waived POC tests to determine an Hgb level are typically based on the azide-methemoglobin method, and most use a drop-size sample of capillary, venous, or arterial blood. One POC device, the Alere HemoPoint H2 Meter, uses optical absorption photometry to obtain Hgb and Hct from a single drop of blood in less than one minute.

In addition to the POCT devices utilizing blood samples, a noninvasive, multiwavelength sensor method utilizing a spectrophotometric method has been developed to determine Hgb concentration.[38] The Masimo SpHb device uses a sensor that emits wavelengths of light to measure Hgb concentration data based on light absorption through the finger. In a similar manner as conventional pulse oximetry, it uses signal processing algorithms and adaptive filters to translate the absorption data. The proposed advantages of a noninvasive testing device would be no risk of exposure to bloodborne pathogens for the pharmacist and a painless process for the patient.

Physiological factors can affect Hgb measurement, emphasizing the need for consistency in laboratory procedures and documentation. Identified causes of variation include capillary blood versus venous blood; tourniquet use for longer than 30 seconds; whether the patient is standing, sitting, or supine; the time of day; whether the right or left hand is used; and even which finger is used for a capillary sample.[39]

HIV

There are several CLIA-waived POC tests to detect antibodies to HIV-1 and HIV-2, but the Alere Determine HIV-1/2 Ag/Ab Combo also reacts to HIV-1 p24 antigens. The time-to-test results for HIV POC tests, following specimen collection, ranges from 1 to 20 minutes, which enables a patient to receive pretest and posttest counseling within a single visit. These test devices are typically based on a capillary lateral flow design and

TABLE 4-1. Summary of CLIA-Waived POC Tests for HIV and HCV[a]

TEST	MANUFACTURER	CLIA WAIVER GRANTED	METHOD	ANALYTE(S)	SPECIMEN TYPE	TIME TO RESULTS (MIN)	OVERALL CLINICAL SENSITIVITY (%)[b]	OVERALL CLINICAL SPECIFICITY (%)[b]
HIV								
Clearview Complete HIV 1/2	Alere	Mar 2011	Lateral Flow	HIV-1/2 Abs	FSWB; VPWB	15	HIV-1 Abs = 99.7 HIV-2 Abs = 100	99.9
Determine HIV-1/2 Ag/Ab Combo Test[40]	Alere	Dec 2014	Lateral Flow	HIV-1 p24 Ag HIV-1/2 Abs	FSWB	20	HIV-1 Abs = 99.9 HIV-2 Abs = 100	99.7
INSTI HIV-1/2 Antibody Test[41]	BioLytical	Jul 2012	Flow Through	HIV-1/2 Abs	FSWB	1	99.8[c]	99.5
OraQuick Advance Rapid HIV-1/2 Antibody Test	OraSure Technologies	Jun 2004	Lateral Flow	HIV-1/2 Abs	OMT; FSWB VPWB	20	HIV-1 Abs 99.3 (OMT) 99.6 (FSWB)	99.8 (OMT) 100 (VPWB)
Uni-Gold Recombigen HIV Test	Trinity Biotech	Nov 2004	Sandwich	HIV-1/2 Abs	FSWB VPWB	10	100	99.7
HCV								
OraQuick Rapid Antibody HCV Test	OraSure Technologies	Feb 2011	Lateral flow	HCV Abs	FSWB VPWB	20–40	[d]	[d]

Abs = antibodies; Ag = antigen; CLIA = Clinical Laboratory Improvement Amendments; FSWB = whole blood via fingerstick; HCV = hepatitis c virus; HIV = human immunodeficiency virus; min = minutes; POC = point of care; OMT =oral mucosa transudate; VPWB = whole blood via venipuncture.

[a] Values obtained from product package inserts and manufacturer websites, except where noted (references 40 and 41).

[b] Data for whole blood via finger stick unless otherwise noted; data for whole blood via venipuncture not provided.

[c] Only HIV-1 Abs data provided.

[d] FSWB positive percent agreement = 97.9%; negative percent agreement = 98.5%; VPWB positive percent agreement = 99.5%; negative percent agreement = 99.0%.

use whole blood from a finger stick or oral mucosal transudate. The performance characteristics of these tests are summarized in **Table 4-1**. In general, the HIV POC tests have sensitivity and specificity equivalent to nonwaived HIV screening test kits (e.g., enzyme-linked immunosorbent assay [ELISA]) approved for laboratory use.[42]

The performance characteristics of the CLIA-waived HIV tests are sufficient to detect HIV infection or its absence. However, these tests are less sensitive than the nonwaived laboratory-based ELISAs and automated systems for detecting acute infections (e.g., seroconversion).[42] In addition, during acute infection, assays performed on oral mucosal transudate have lower sensitivity than tests performed on whole blood, serum, or plasma because the antibody concentration found in oral mucosal transudate is lower.[43,44] The HIV antibody titers are low in acute HIV infection, and although acute HIV is difficult to detect with CLIA-waived POC tests, the tests of oral mucosal transudate often miss such infections because of lower sensitivity.[42,44] Combination tests that measure p24 antigen in addition to antibodies for HIV-1 and HIV-2 may allow for early detection of infection.[40] Also, HIV prevalence in the population being tested can impact the oral tests.[43] In high-prevalence settings, the PPVs for either specimen type are similar; however, in low-prevalence settings, PPVs are much higher for blood than oral mucosal transudate specimens.[44]

HCV

Currently, there is one FDA-approved CLIA-waived POC test—the OraQuick HCV Rapid Antibody Test—that detects antibodies to HCV from whole blood samples obtained either by venipuncture or finger stick. Although it is not yet approved for oral specimens in the United States, this test can also detect anti-HCV antibodies from oral mucosal transudate.[45] The results from can be read between 20–40 minutes after the analysis is started, which enables a patient to receive pretest and posttest counseling within a single visit. This test is a noninstrumented, indirect lateral flow immunoassay, and its performance characteristics are summarized in Table 4-1. It has excellent sensitivity and specificity using whole blood from a finger stick.[46] In a multicenter study of individuals at risk for HCV infection, the test demonstrated clinical performance equivalent to laboratory-based tests across all specimen types.[47] In addition, several studies have observed that the specificity of the HCV rapid test with all specimen types is similar to that reported for anti-HCV enzyme immunoassay (EIA).[48] The CLIA-waived POC test to detect antibodies to HCV also has a high degree of interoperator agreement in result interpretation.[49] For these reasons, the OraQuick HCV Rapid Antibody Test can be used for initial HCV antibody screening as an alternative to the third-generation EIA methods.[46]

Influenza A and B

The majority of marketed CLIA-waived POC influenza diagnostic tests use chromatographic immunoassay technology to detect the influenza virus nucleoprotein antigen. Recently, technological advances like the advent of isothermal nucleic acid amplification methods that enable rapid detection of DNA/RNA have made POC molecular diagnostic testing possible.[50] In 2015, the Alere Influenza A & B Test became the first molecular test to receive a CLIA-waived designation. This test uses an isothermal nucleic acid amplification method to qualitatively detect and differentiate influenza A and B viral RNA. As clinical experience with molecular-based POC tests increases, it is likely that more such tests will be developed. In the future, these tests may prove useful for population-based screening of asymptomatic infectious diseases or rapid detection of common pathogens such as GAS.[51,52]

The marketed CLIA-waived POC influenza diagnostic tests provide results within 15 minutes; thus, they are commonly used to diagnose influenza in the ambulatory care clinics.[53,54] The performance characteristics of marketed CLIA-waived POC influenza tests are summarized in **Table 4-2**. All CLIA-waived POC influenza tests are designed as lateral flow immunoassays, and as mentioned above, all but one contain antibodies specific to the nucleoprotein of influenza A and B viruses.[53,54] Qualitative detection of each virus type occurs via a visual colorimetric signal or requires a reader to interpret reflectance or fluorescence.[54] With the exception of the molecular test, reactivity is strongly correlated to nucleoprotein antigen concentration; thus, sensitivity is higher with increasing concentration and diminished at lower concentrations. As discussed below, this variability should be considered when collecting samples and interpreting negative results. To maximize viral antigen concentration in the sample, clinicians should collect test samples using methods outlined in the manufacturer's test package insert and as stipulated in the CLIA regulations.

Group A Streptococci

Group A streptococci (GAS) is the most common bacterial cause of acute sore throat, but it is difficult to accurately diagnose it as a cause of pharyngitis.[55] On the basis of clinical manifestations alone, GAS pharyngitis is often indistinguishable from viral etiologies.[52,55] In contrast, to adults, GAS pharyngitis is more common than viral etiologies among children and adolescents. Therefore, to aid in the identification of patients with pharyngitis who have a high likelihood of GAS infection, validated age-based clinical prediction rules, which are based on a combination of signs and symptoms like the Modified Centor Criteria score, can be used.[56] Originally developed in 1981, the Modified Centor Criteria score was derived from a study of 286 adults with a sore throat who presented to an emergency department.[56] Initially it was based on the presence of four signs and symptoms—tonsillar exudates, swollen tender anterior cervical nodes, fever, and the lack of cough—and used to estimate the probability of acute GAS pharyngitis in adults with sore throat.[56] The score was later modified by adding age to the four criteria and validated in a larger study of adults and children.[57] The Modified Centor Criteria score is based on a total score that determines the likelihood of GAS pharyngitis.[57] It assigns one point each of the four signs and symptoms and age <15 years; no point for age is assigned for ages between 15 and 44 years, and a point is subtracted for age >44 years.[52,57] Scores range from –1 to 5; patients with a score of 2 or higher are recommended to be tested for GAS.[52] In 2004, the risk of GAS pharyngitis based on the Modified Centor Criteria was further refined, but the criteria to determine the score remained the same.[58] Even when using this clinical prediction rule, clinicians have limited success in accurately diagnosing GAS pharyngitis because only 53% of patients with GAS pharyngitis are identified at the highest Modified Centor Criteria scores (e.g., ≥4).[52, 55-58]

POC tests that detect GAS antigens have been marketed for years and have evolved and improved through several generations. Today, numerous tests use a variety of antigen detection methods (e.g., latex agglutination, ELISAs, lateral flow immunochromatographic assays, and optical immunoassays), but not all are CLIA-waived. The use of POC tests has been incorporated into the current Infectious Diseases Society of America (IDSA) guidelines for the diagnosis and management of GAS pharyngitis.[59] Because POC tests for GAS infection have demonstrated limited sensitivity, IDSA guidelines recommend confirming negative results in populations at high-risk of developing pharyngitis because of GAS or viral etiologies with a throat culture to reduce the chance of missing a positive case.[59] The specificity associated with POC tests for GAS infection is high; therefore, positive results do not require a backup culture in a patient, regardless of age.[59]

Recently, a comprehensive meta-analysis of 48 studies found that sensitivity and specificity across all studies analyzed was 86% and 96%, respectively.[55] With the exception of molecular-based methods, which are not yet CLIA-waived, the sensitivity and specificity were comparable across assay methods.[55] Investigators also found that although there was marked variability in sensitivity, specificity varied little across studies.[55] Overall, the study demonstrated that the sensitivity of POC tests for GAS is sufficiently high and a backup culture is not needed, particularly given the low risk of complications such as acute rheumatic fever in the United States.[55] Moreover, the investigators concluded that the high overall specificity of POC tests for GAS could minimize the overdiagnosis of GAS pharyngitis and prevent unnecessary antibiotic use in such cases.[55]

Applying Test Performance Characteristics to Testing Practices

Accuracy/Bias and Precision

These are two performance characteristics that are important to the quality control processes of any CLIA-waived POC test. All tests have variabilities that are random and systematic associated with their use. Performance characteristics,

TABLE 4-2. Summary of Performance Characteristics for CLIA-Waived POC Tests for Influenza A and B[a]

TEST (MANUFACTURER)	INFLUENZA TYPE	SPECIMEN TYPE	TIME TO RESULTS (MIN)	OVERALL CLINICAL SENSITIVITY (%)[e] A/B		OVERALL CLINICAL SPECIFICITY (%)[e] A/B		PPV (%)[e] A/B		NPV (%)[e] A/B		OVERALL ACCURACY (%)[e] A/B	
Influenza	---	---	---	NS	NPS	NS	NPS	NS	NPS	NS	NPS	NS	NPS
Influenza A&B Test (Alere)	A and B	NS	10	94/77	---	96/98	---	95/82	---	96/98	---	99/95	---
Alere i Influenza A & B[b] (Alere)	A and B	NS	15	97.9%/92.5[f]	---	86.2/96.5[f]	---	94.8/98.4	---	97.7/99.4	---	97/99	---
Veritor System—CLIA-waived for Rapid Detection of Flu A+B (Becton Dickinson)[b]	A and B	NS, NPS	10	---	---	---	---	82.1/74.6	74.6/73.3	98.6/99.6	96.4/99.4	[g]	[h]
BinaxNOW Influenza A&B Card (Alere)	A and B	NS, NPS, NA, NW	15	93.8/77.4	---	95.8/98	---	95.2/82.5	---	96.1/98.4	---	---	---
QuickVue[c] Influenza (Quidel Corp)	A and B	NS, NA, NW	≤10	73	---	96	---	92	---	85	---	87	---
QuickVue Influenza A+B Test (Quidel Corp)	A and B	NS, NPS, NA, NW	≤10	94/70	83/62	90/97	89/98	62/82	67/80	99/94	95/95	91/93	88/94
SAS FluAlert Influenza A (SA Scientific)	A only	NW	15	[i]	[i]	[i]	[i]	[i]	[i]	[i]	[i]	[i]	[i]
SAS FluAlert Influenza B (SA Scientific)	B only	NW	15	[i]	[i]	[i]	[i]	[i]	[i]	[i]	[i]	[i]	[i]
Sofia Influenza A+B FIA[b,d] (Quidel)	A and B	NS, NPS, NA, NW	15	90/89	97/90	95/96	95/97	---	---	---	---	---	---

FIA = fluorescent immunoassay; IFVA = influenza virus A; IFVB = influenza virus B; min = minutes; NA = nasal aspirate; NP= nucleoprotein; NPV = negative predictive value; NPS = nasopharyngeal swab; NS = nasal swab; NW = nasal wash; PPV= positive predictive value.

[a] Values obtained from product package inserts and from manufacturer websites.

[b] Requires separate reader device.

[c] Does not distinguish between influenza A and B virus infections when used alone.

[d] Immunofluorescence assay.

[e] Data for nasal aspirate and nasal wash not included.

[f] Koski RR, Klepser ME. A systematic review of rapid diagnostic tests for influenza: considerations for a community pharmacist. *J Am Pharm Assoc* 2017;57:13–19.

[g] A/B positive percent agreement of all swabs (NS or NPS) = 82.1/79.7

[h] A/B negative percent agreement of all swabs (NS or NPS) = 98.1/99.4.

[i] Manufacturer website does not provide data for NS or NPS.

such as *accuracy/bias* and precision, help the clinician choose the test; compare the expected performance of a test across different manufacturers; evaluate the validity of its results; and assess whether the test is performing within its expected error limits. These performance characteristics are determined in studies for regulatory approval; the results of which are included in the manufacturer's provided package insert with the individual test. Although similar, accuracy/bias and precision describe different performance characteristics. A test can be accurate, but not precise; or precise, but not accurate.

Sensitivity and Specificity

Sensitivity and *specificity* are performance characteristics that are used to evaluate a test. In general, these values are independent of the population of interest being tested and can be considered fixed characteristics of the test.[23] Therefore, their values do not significantly change unless the test is further optimized with improvements in methodology or analytical techniques. When a clinician seeks a test to screen for a condition or disease they know the majority of their patients will not have, they want the method with the highest sensitivity to optimize testing efforts. A test that is highly sensitive means it does not often produce a false-negative result, if at all. By choosing the method with the highest sensitivity, the clinician minimizes the chance that someone with the condition or disease goes undetected. Likewise, by choosing the method with the highest specificity, the clinician minimizes the chance that someone without the condition or disease will be misidentified as having it. In assessing the value of sensitivity and specificity and relating them to patients in their clinical setting, clinicians also must consider the test's PPV and NPV.[23]

PPVs and NPVs

These values provide useful insight into how to interpret test results. As discussed above, these values are dependent on the population being tested and are influenced by the disease prevalence. Thus, knowledge of how these values are influenced by disease prevalence can help clinicians optimize test performance and mitigate needless testing. The PPV allows the clinician to answer the question from the concerned patient who tested positive, "how likely is it that I have this condition," whereas the NPV allows the clinician to determine how reassuring to be in answering the question from the patient who tested negative, "how likely is it that I do not have this condition."[23]

LIMITATIONS OF POCT

Legal and Regulatory Barriers to Pharmacists Performing CLIA-Waived POC Tests

Under the CLIA requirements, there are no minimum educational or training requirements needed for the director or testing personnel.[3,4] Therefore, in nearly all states pharmacists can serve in either of these roles and perform a CLIA-waived POC test. However, the ability of pharmacists to use the result of a CLIA-waived POC test to make a therapeutic decision for the management of chronic diseases or acute infections falls under the scope of practice, which is regulated by state agencies and boards of pharmacy.[2] However, studies have identified that legislative and regulatory variability across states may produce confusion among practitioners and represent a barrier to pharmacists' efforts to perform POCT.[2,60] Although performing CLIA-waived POCT may not be explicitly addressed in many pharmacy practice acts, such activities may be permissible under CPA or CDTM provisions in state regulations or statutes.[2] A few states, however, still do not have CPA/CDTM provisions in their pharmacy practice acts. There is variability across states with CPA/CDTM provisions in terms of specificity, scope, and structure.[2] For example, some states restrict CPA/CDTMs to written agreements for individual patients, whereby pharmacists can treat a patient for the condition specified in the protocol only if they have advanced authorization from the patient's primary care provider. Such an approach poses a challenge for patients who do not have a primary care provider.[61]

Lack of Training/Education

According to their classification, CLIA-waived POC tests are simple to perform with a low risk for erroneous results or harm to patients if performed incorrectly.[3] However, they are not error-proof; CLIA regulations explicitly stipulate that those performing the tests must strictly adhere to the manufacturer's instructions.[3] Although CDC and CMS studies indicate that waived laboratories (including pharmacies) generally perform testing correctly, the results of the agencies' surveys also highlight the need for additional education and training for site directors and testing personnel.[4] Because CLIA requirements do not specify any level of education for directors or testing personnel in waived laboratories, lack of education regarding CLIA-waived testing is a gap that exists across all healthcare professional education.[4] Two national surveys of academic pharmacy suggest that education on CLIA-waived POC tests for infectious diseases is generally lacking from professional pharmacy degree programs.[62,63] This is an opportunity for expansion of core curricula that would enhance pharmacists' participation in POCT services.

GOOD LABORATORY PRACTICES

Although CLIA-waived tests are determined to be simple for the user, it is necessary for a pharmacist to be properly trained on each CLIA-waived device and for the laboratory to follow "Good Laboratory Practices" for waived testing sites per CDC guidelines.[4] These practices include steps that should be taken before initiating or expanding CLIA-waived POCT services and include actions taken during and after the actual performance of test.[4]

Before Initiating Services (Preparation Phase)

Recommended practices prior to initiating POCT testing services involve regulatory, logistic, procedural, and personnel considerations. An initial step toward initiating CLIA-waived POCT services is to identify a qualified individual who will be responsible and accountable for testing operations.[4,6] As described above, some states have additional requirements that must be met to fulfill this role. Individuals leading and working in a waived laboratory must be familiar with local, state, and federal regulatory requirements. In addition to the CLIA requirements and state pharmacy practice acts, practitioners need to be familiar with state and local regulations governing laboratory operations, federal laws governing privacy (e.g., HIPAA [Health Insurance Portability and Accountability Act]), Occupational Safety and Health Administration (OSHA) work place safety standards (e.g., OSHA standards related to workplace hazards and Bloodborne Pathogens Standards), and information from the CDC and CLSI regarding biosafety and precautions for preventing transmission of bloodborne pathogens in the workplace.[4,6] Having familiarity with these regulations and resources will help ensure that any CLIA-waived POCT services protect patient confidentiality and are safe for both the patient and testing personnel.[4]

Many factors must be considered prior to initiating services to create a testing space that meets the needs of the practice setting, employees, patients, and the environmental requirements specified in the test manufacturers' package insert. Additional considerations that are addressed include a fiscal assessment of the proposed POCT services and an analysis of offered tests so that all factors required to properly conduct the test(s) of interest can be determined (e.g., the advantages and disadvantages of the available devices, any additional equipment, and access to ancillary care services).[4]

The development of written policies and procedures that clearly outline the responsibilities and testing instructions for testing personnel and facility directors are a critical process that must occur before testing begins. Such procedures should be based on the manufacturer's instructions and be used to train testing personnel.[4] In addition to test performance, the policies and procedures should outline and standardize specimen collection techniques; quality control procedures; proper handling and storage of tests and reagents; and documentation and reporting of results.[4]

The testing personnel are a critical component to any CLIA-waived POCT service. Because personnel at waived testing sites are not subject to proficiency testing, it is essential that they be trained by a qualified person and be competent in any test they will perform prior to performing the test.[4] Training should include an observed performance of the trainee performing the test. There are many training resources available through test manufacturers and distributors, professional organizations, and governmental agencies. Training should be documented and reviewed on a regular basis to ensure that all updates or changes are noted. Although proficiency testing is not mandated, including such assessments is recommended as part of any quality assurance programs.[4]

Test Ordering and Sample Collection (Preanalytical Phase)

Having considered all of the above aspects in the planning phase helps ensure personnel have the resources, understanding, and skills needed to properly perform a CLIA-waived POC test during the preanalytical phase. Good laboratory practices in this phase include confirming the test has been properly ordered; identifying the patient; labeling the sample collection device to avoid any confusion with other patients; providing the patient with pretest education or information; reviewing the complete test procedure; and preparing the test area and materials.[4]

During Testing (Analytical Phase)

Good laboratory practices in the analytical phase involve quality control (QC), test performance, and result interpretation and documentation.[4] The QC measures are important to ensure proper training of the user's technique, the integrity of the testing device, and the overall performance of the POCT analytical device. It is a process that consists of two components, internal QC and external quality assessment (QA). Internal QC requires the analysis of manufacturer provided QC materials. These materials are imbedded in the test in known concentrations and produce a result that indicates whether the analytical method and the reading device are functioning properly. The purpose of internal QC is to monitor the precision and function of the analytical method over time. In contrast, external QA monitors the testing process from specimen application to result interpretation. External QA requires analyzing patient-like samples comprised of liquid or other materials similar to patient specimens provided by the manufacturer or purchased separately. These patient-like samples can be run prior to or concurrent with patient samples, and their results are compared across testing personnel to monitor the accuracy of reporting. The QC test results should be documented and any action taken in response to the QC tests should be recorded. The CLIA-waived laboratory director should incorporate a QC plan in the site's policy and procedures.[4] The POC test and device manufacturer will provide QC materials, which often includes internal and external controls. A test site should determine a QC testing frequency that fits its operations for each test system, but at a minimum QC testing should be performed as often as the product insert recommends.[4]

Each CLIA-waived POC test and device can require different techniques for acquiring or testing a sample. Therefore, CLIA requirements stipulate that when personnel perform the tests they must strictly adhere to the manufacturer's guidelines and follow specific storage conditions for the test and test materials (e.g., testing strips, cartridges, cassettes, and reagent). The temperature and humidity may also be critical factors in providing a successful test result. The CLIA-waived POC test results should be interpreted within the manufacturer's specified time period, and the test should be repeated if the results conflict with the available clinical information or are invalid.[4] Once valid test results are obtained, they should be documented according to established policy and procedures in a timely fashion.

After Testing (Postanalytical Phase)

Good laboratory practices in the postanalytical phase involves issuing test reports, performing supplemental or confirmatory testing, testing area cleanup, disposing biohazard waste, and documenting testing activities. The pharmacist's appropriate interpretation of the test results is a critical part of the postanalytical phase, as well as communicating the results to the patient and provider. The pharmacist's discussion of a laboratory result with the patient should be well planned and considerate of the patient's response. When a pharmacist encounters a test result that requires follow-up with a physician for further evaluation, it is important to communicate with the patient's physician and have referral resources ready and available as needed. In some cases, good laboratory practices and state or local statutes mandate that test results for certain infectious diseases also should be reported to local or state public health agencies.[4] For all postanalytical phase activities, the testing site should have specific policies and procedures clearly defined and in place.

APPLYING CLIA-WAIVED POC TESTS TO PATIENT CARE IN A PHARMACY PRACTICE SETTING

The Pharmacists' Patient Care Process, adopted by the Joint Commission of Pharmacy Practitioners provides a patient-centered approach in collaboration with other healthcare providers to optimize patient health and medication outcomes. Nationally, pharmacist involvement in chronic disease state management, through formalized routes such as medication therapy services, CDTMs, or CPAs, is becoming increasingly prevalent. The use of CLIA-waived POC tests provides another tool for pharmacists working in a patient-centered, team-based environment to contribute to the appropriate management of chronic disease. The CLIA-waived POCT services can be used in a community pharmacy or ambulatory care clinic to monitor the safety, efficacy, and adherence of medications, as well as provide measurable clinical outcome data consistent with evidence-based guidelines.

Determining Appropriate Testing Candidates

To provide consistency in the patient care process, it is vital to develop appropriate policies and procedures specific to each CLIA-waived POC test to optimize the ability of the test to produce results that aid in chronic disease state management or detect the analyte of interest without overtesting. For CLIA-waived POC tests in chronic disease state management, clinicians should specify the frequency of testing as part of the patient-specific care plan in accordance with relevant national clinical guidelines. In addition, frequency of monitoring should be driven by the patient's disease progression and achievement of treatment goals.

Because CLIA-waived POC tests for infectious diseases are qualitative and used to screen for infections of public health interest or to aid in the diagnosis and management of an acute infection in an individual patient, overtesting is a concern. To optimize the ability of the test to detect the analyte of interest and avoid overtesting, clinicians should develop practices that enable them to distinguish patients who could benefit from testing. As described previously, low disease prevalence may negatively impact the PPV of qualitative tests. Therefore, to optimize test performance and minimize overtesting, clinicians must perform relevant physical assessments to gather additional information and identify patients who need testing (e.g., make referrals to their physicians for follow-up, or make immediate referrals to emergency medical care).

Current CLIA-waived POC tests for infectious diseases detect pathogens that are seasonal (e.g., influenza), produce symptomatic infections that are difficult to diagnose based on symptoms alone (e.g., GAS and influenza), or produce asymptomatic infections that are most prevalent in specific high-risk populations (e.g., HIV and HCV). To properly use these tests and mitigate overtesting, additional strategies may be required.

Influenza A and B

A concern with some CLIA-waived POC tests for influenza has been inconsistent accuracy, characterized by highly variable sensitivity, despite very high specificity.[64] A large meta-analysis evaluated 159 studies (20 of which were with POC tests) involving the comparison of 26 rapid influenza diagnostic tests to a reference standard (reverse transcriptase-PCR or culture).[64] Included in the meta-analysis were 55 studies conducted during the 2009 H1N1 pandemic.[64] The study determined pooled sensitivity and specificity were 62.3% and 98.2%, respectively.[64] In general, the tests were more sensitive for influenza A than B and had lower sensitivity in adults than in children. Although there was concern about the sensitivity of POC tests during the 2009 H1N1 pandemic, this meta-analysis found no difference in the performance characteristics of the POC tests between studies conducted during the 2009 H1N1 pandemic and those conducted before the outbreak.[64] The accuracy of POC tests were not different than when trained laboratory personnel performed the test. A meta-analysis of fewer studies that evaluated the performance of POC tests during the 2009 H1N1 pandemic also found the tests had low sensitivity (51%) and high specificity (98%).[65] In addition, some CLIA-waived POC tests had limited ability to detect novel strains of influenza A (H7N9).[66]

The seasonal variability in influenza activity directly affects the predictive values of CLIA-waived POC tests for influenza. Given their high specificity and modest and highly variable sensitivities, clinicians can trust a positive POC test result during a flu season in a patient demonstrating an influenza-like illness and initiate appropriate management. However, when a POC test for influenza is negative, there is a good likelihood that it may be a false negative. The CDC has published several useful algorithms to guide when to perform CLIA-waived tests for influenza for individual patients when influenza viruses are circulating in the community. They also have published useful algorithms to aid in the interpretation of test results when influenza viruses are circulating or not circulating in the community, or when influenza activity is low in the community.[67] Accordingly, testing should be performed using a CLIA-waived POC test that is highly sensitive and specific during the influenza season when the disease prevalence is high. Moreover, such testing should be guided by an appropriate physical assessment to determine if the patient has symptoms consistent with an acute respiratory disease that began within several days of patient presentation.[67]

In a pilot study, pharmacy researchers developed a physician–pharmacist collaborative management model for influenza based largely on the CDC principles.[68] In that model, pharmacists in a community practice setting screened all adults with symptoms consistent with an influenza-like illness. Using proper specimen collection technique, nasal swabs collected from individuals were tested by a CLIA-waived POC test for influenza.[68] The PPV of the tests was maximized by performing these activities only when local influenza activity had been documented by state or federal surveillance and by performing a physical assessment and assessing vital signs (e.g., heart rate, blood pressure, respiratory rate, temperature, and oxygen saturation).[68] Only 11% of patients with an influenza-like illness had a positive result, and because the CPA prevented pharmacists from dispensing oseltamivir or antibacterial therapy to patients who tested negative, the study demonstrated that this practice model can lead to rationale use of antivirals and avoid the overuse of unnecessary antimicrobial therapy.[68]

To determine whether influenza is present in a specific patient population and assist providers in diagnosing and treating acute respiratory illnesses, the CDC also recommends that CLIA-waived POCT be done during an acute outbreak of a respiratory disease and in patients with clinical signs and symptoms of influenza during the influenza season.[67]

GAS

When performing CLIA-waived POCT for symptomatic infections that are difficult to diagnose based on symptoms alone (e.g., GAS and influenza), limiting testing to certain time periods will certainly improve the PPV of CLIA-waived POC tests for those pathogens, like influenza, that are seasonally distributed. **(Minicase 1.)** However, other strategies are needed for nonseasonal distributed pathogens such as GAS. Performing an appropriate physical exam and applying its results using the validated, age-based Modified Centor Criteria, the clinical prediction rule may further assist in identifying those patients with pharyngitis who would benefit most from CLIA-waived POCT for GAS. In a pilot study, pharmacy researchers developed a physician–pharmacist collaborative management model for GAS based largely on these principles.[69] In that model, pharmacists in a community practice setting screened 316 patients, of which 273 were eligible for testing. Only 48 patients (17.5%) had a positive test result and received amoxicillin or azithromycin per their CPA. This pilot project demonstrates that such a practice model can dramatically reduce inappropriate antimicrobial use in the community practice setting.[69]

MINICASE 1

Group A Streptococcal Pharyngitis and POCT

Maya B., a 10-year-old girl, presents to the community pharmacy in November with her mother. The mother states Maya B. began complaining of a sore throat, mild cough, and headache after attending a slumber party two days prior. In addition, one of the other girls attending the slumber party tested positive for GAS yesterday. Maya B.'s physical findings include a red throat and tender cervical lymph nodes.

QUESTION: What additional information should the pharmacist obtain to determine if CLIA-waived POCT for GAS would be appropriate in this patient?

DISCUSSION: The pharmacist should obtain vital signs and calculate a Modified Centor Criteria score as part of the CPA to determine the likelihood of GAS pharyngitis.

Vital signs: BP 120/70 mm Hg, HR 80 beats/min, RR 20 breaths/min, temperature 101 °F, and weight 90 lb.

Modified Centor Criteria Score[52,56-58]	Patient's Calculated Score
Absence of cough (+1)	(0)
Swollen and tender anterior cervical lymph nodes (+1)	(+1)
Temperature >100.4 °F (+1)	(+1)
Tonsillar exudate or swelling (+1)	(0)
Age (years):	(+1)
3–14 (+1)	Score = 3; probability of GAS ~28–35%
15–44 (0)	
>45 (–1)	

QUESTION: Based on the pharmacist's findings, how should the pharmacist proceed under CPA?

DISCUSSION: The pharmacist should perform a throat swab and CLIA-waived POCT to verify the presence of GAS. The recommended management of GAS according to the patient's Modified Centor Criteria score would be antibiotics based on the result of the CLIA-waived POC test. In addition, the pharmacist should recommend an analgesic/antipyretic for symptom management.

HIV and HCV

When performing CLIA-waived POCT for asymptomatic infectious diseases that are most prevalent in specific high-risk populations like HIV and HCV, refer to national guidelines to determine who should be tested.[9,70]

Chronic Disease State Management Applications of CLIA-Waived POC Tests

As part of value-added services, pharmacists in a community pharmacy or an ambulatory care clinic can build CLIA-waived POCT services into their workflow. As described previously, common CLIA-waived POC tests for chronic disease state management require collection of a small blood sample for the monitoring of diabetes, blood glucose, HgbA1C, or creatinine. **(Minicase 2.)** Depending on the state board of pharmacy's statutes, the pharmacist engaged in a CPA/CDTM could then follow the protocol to adjust medication dosages, recommend nonpharmacological therapies, or schedule additional testing or physician follow-up. Patients with diabetes and cardiovascular disease often take renally eliminated medications and could benefit from monitoring of renal function through a CLIA-waived POCT services for creatinine.

Population/Public Health Applications of CLIA-Waived POC Tests for Infectious Diseases

Studies show that the accessibility of community pharmacies can be exploited to successfully offer CLIA-waived POCT services for HIV infection.[71-76] Moreover, establishing CLIA-waived POCT services for HIV in a pharmacy practice setting requires a modest amount of staff training, and the costs are similar to other services offered in these settings.[72-74, 76] Collectively, studies indicate there is an relatively untapped potential for pharmacies to serve as an alternative, highly accessible, and less stigmatizing healthcare facility to perform HIV testing services, which could increase testing rates in the United States.[71-76]

Similar to HIV, it is likely that increasing access to testing through CLIA-waived POCT services in community and ambulatory pharmacy practice settings could help identify patients infected by other pathogens and hasten their access to care and appropriate therapies. Providing POCT services for influenza in community and ambulatory pharmacy practice settings represents another possibility for pharmacists to collaborate with public health agencies to improve seasonal surveillance efforts and reduce inappropriate antibacterial use in respiratory illnesses during the influenza season.[2,61]

Individual/Patient-Specific Applications of CLIA-Waived POC Tests for Infectious Diseases

Performance characteristics of current CLIA-waived POC tests for GAS are sufficiently robust that current guidelines do not recommend any additional confirmatory tests for adults when results are negative. Even though confirmatory testing is currently recommended when CLIA-waived POC test results are negative in children and adolescents, data suggest such follow-up testing may not be necessary.[55,59]

Seasonal influenza is another acute respiratory illness that is challenging to diagnose by clinical signs and symptoms. In addition, there is a narrow period of time during which initiating antiviral therapy can produce a beneficial outcome. Providing CLIA-waived POCT services for seasonal influenza and GAS in ambulatory care clinic or community pharmacy practice settings may augment physical assessments and, when performed under a CPA/CDTM, could allow standard therapy to be promptly initiated, which may also reduce inappropriate antimicrobial use in acute respiratory illnesses.[61]

MINICASE 2

Type 2 Diabetes Mellitus and HgbA1C

Stuart M., a 60-year-old male with a five-year history of type 2 diabetes mellitus, presents to his primary care clinic for a follow-up visit. Although he was diagnosed five years earlier, he exhibited symptoms of diabetes for at least two years before diagnosis including nocturia. He reports increased physical activity but has gained 10 pounds over the past year with little success in weight loss (weight 190 lb; height 5′10″; BMI 27.3 kg/m^2). He reports eating a high-carbohydrate diet with pasta or bread at every dinner.

Stuart M.'s medications for diabetes include metformin 500-mg tablets, one tablet twice a day for the past four years, and various nutritional supplements that he has tried with no noticeable improvement. His personal blood glucose logs over the past three months indicate values ranging throughout the day from 108–264 mg/dL. Previous A1C level was 7.8% tested six months ago at the clinic. The pharmacist performs POC A1C testing based on a CPA. Stuart M.'s vital signs include BP 140/85 mm Hg, HR 84 beats/min, RR 20 breaths/min, and HgbA1C: 8.2%.

QUESTION: What does this A1C level indicate?

DISCUSSION: The rise in A1C level of 0.4% over six months indicates that the patient's average blood glucose level is increased, and he is at increased risk for diabetes complications. In addition to the A1C level, the patient's weight gain and report of a high-carbohydrate diet would contribute to the pharmacist's decision to modify therapy. According to the CPA, the pharmacist might decide to maximize the metformin therapy, along with educating the patient on dietary strategies to reduce the amount of carbohydrates consumed and increasing physical activity with the goal of weight loss. The pharmacist might also recommend a follow-up visit with a repeat A1C level in three months.

FUTURE APPLICATIONS FOR CLIA-WAIVED POC TESTS IN COMMUNITY AND AMBULATORY PHARMACY PRACTICE SETTINGS

Provided POCT services fit the workflow of the pharmacy practice setting and are fiscally sound with the continual scientific advances, transformations in the delivery of healthcare in the United States, and advances in technology, there will likely be many more innovative applications of CLIA-waived POC tests for chronic disease state and infectious diseases in community and ambulatory pharmacy practice settings. Scientific advances in molecular diagnostics will enable the development of new molecular-based CLIA-waived POC tests or improve current antibody/antigen-based tests, which may make it more practical to test infectious disease analytes of public health interest (e.g., tuberculosis and pathogens responsible for sexually transmitted infections). In addition, in cases such as sexually transmitted infections, molecular-based CLIA-waived POC tests could allow a pharmacist working under a CPA/CDTM to institute prompt therapy under protocol.

Ongoing reforms to the U.S. healthcare delivery system will continue to raise awareness of pharmacies as ready access points to the healthcare system. To fully realize this potential for POCT services in community and ambulatory pharmacy practice settings, local, state, and federal regulations governing pharmacy practice must continue to evolve so pharmacists can practice to their fullest professional potential. Technological advances in health informatics will ultimately enable the pharmacist to have access to electronic medical records (EMRs) regardless of practice setting. Similarly, technological advances will ultimately allow the transmission of CLIA-waived POCT results to patients' EMRs, their primary provider, and other relevant public health agencies.

LEARNING POINTS

1. ***How have the Clinical Laboratory Improvement Amendments (CLIA) of 1988 created opportunities for pharmacists in ambulatory care clinics and community pharmacies?***

   ANSWER: Technology has allowed many laboratory tests useful in the detection and management of chronic diseases and infections to be simplified and classified as CLIA-waived. CLIA-waived POC tests represent an opportunity for pharmacists in ambulatory care clinics and community pharmacies to expand their patient care services.

2. ***What must pharmacists in ambulatory care clinics and community pharmacies know to properly perform CLIA-waived tests in their practice settings?***

   ANSWER: To properly perform a CLIA-waived test in ambulatory care clinics and community pharmacies, pharmacists must understand the basis of the test, how to handle specimens, and how to perform the test. They must also understand all relevant state and federal regulations related to performing such tests and reporting the results.

3. ***What must pharmacists in ambulatory care clinics and community pharmacies know to provide useful POCT services in their practice settings?***

   ANSWER: To provide useful POCT services, pharmacists must understand how to identify patients who would benefit from testing. In addition, they must understand how to evaluate the various performance characteristics and the limitations of the test.

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5

SUBSTANCE ABUSE AND TOXICOLOGICAL TESTS

Peter A. Chyka

OBJECTIVES

After completing this chapter, the reader should be able to

- **List the general analytical techniques used in substance abuse and toxicological screening and discuss their limitations**
- **Compare the uses of preliminary and confirmatory urine drug tests**
- **Discuss the considerations in interpreting a positive and negative drug screen result**
- **Discuss why interfering substances can cause false-negative and false-positive results of screening tests**
- **Recognize the uses of serum drug concentrations in the evaluation and treatment of a patient who has a suspected poisoning or overdose**
- **Describe how the pharmacokinetics of a drug in overdose may affect the interpretation of serum concentrations**
- **Discuss how toxicologic analyses may be helpful in medicolegal situations, postmortem applications, athletic competition, and prescription drug abuse**

When substance abuse, poisoning, or overdose is suspected, the testing of biological specimens is crucial for characterizing usage or exposure, monitoring therapy or abstinence, or aiding in diagnosis or treatment. Millions of Americans are potentially subject to these types of tests. According to the 2014 National Survey on Drug Use and Health, 27 million Americans aged 12 years and older (10.2% of the population) reported using an illicit drug in the past month and 44.2 million (16.7%) reported illicit drug use during the past year (**Table 5-1**).[1] Poison control centers document approximately 2 million unintentional and intentional poison exposures each year with one half occurring in children under six years of age, approximately 90% of cases occurring in homes, and two thirds managed onsite in a nonhealthcare facility (**Table 5-2**).[2]

Illicit drug use during 2014 has been estimated to involve 17.4 million part-time and full-time workers aged 18 years and older with another 12 million claiming heavy alcohol use (five or more drinks per occasion on five or more days).[1] Based on 9.1 million urine drug screens performed by a nationwide laboratory service in 2014 for the combined U.S. workforce, 3.9% had positive test results (**Table 5-3**).[3] In 2014, 22.5 million Americans (8.5% of the population) needed treatment for a problem with the use of alcohol or illicit drugs.[1]

TABLE 5-1. Americans Aged 12 Years or Older Reporting Use of Illicit Drugs During 2014[1]

	PERCENTAGE OF THE TOTAL POPULATION	
SUBSTANCE	**PAST YEAR (%)**	**PAST MONTH (%)**
Any illicit drug	16.7	10.2
Marijuana and hashish	13.2	8.4
Cocaine	1.7	0.6
Crack cocaine	0.3	0.1
Heroin	0.3	0.2
Hallucinogens	1.6	0.4
Lysergic acid diethylamide (LSD)	0.5	0.1
3,4-methylenedioxy-N- methamphetamine (MDMA, Ecstasy)	0.9	0.2
Inhalants	0.6	0.2
Any illicit drug other than marijuana	7.4	3.3
Nonmedical use of any		
Psychotherapeutic drug	5.6	2.5
Pain reliever	3.9	1.6
Tranquilizer	2.0	0.7
Stimulant	1.4	0.6
Sedative	0.3	0.1

TABLE 5-2. Ranking of Twelve Most Frequent Poison Exposure Categories Reported to U.S. Poison Control Centers During 2014[a,b]

ALL EXPOSURES	CHILDREN (UNDER SIX YEARS)	ADULTS (OVER 19 YEARS)
Analgesics	Cosmetics, personal care products	Analgesics
Cosmetics, personal care products	Cleaning substances	Sedatives, antipsychotics
Cleaning substances	Analgesics	Antidepressant drugs
Sedatives, antipsychotics	Foreign bodies	Cardiovascular drugs
Antidepressant drugs	Topical drugs	Cleaning substances
Antihistamines	Vitamins	Alcohols
Cardiovascular drugs	Antihistamines	Anticonvulsants
Foreign bodies	Pesticides	Pesticides
Pesticides	Gastrointestinal drugs	Bites, envenomations
Topical drugs	Plants	Antihistamines
Alcohols	Dietary supplements	Cosmetics, personal care products
Vitamins	Antimicrobial drugs	Stimulants

[a] In decreasing order of frequency and based on 2,577,577 substances reported in 2,165,142 cases.
[b] Data from reference 2.

TABLE 5-3. Rate of Positive Urine Drug Screens for the Combined U.S. Workforce During 2014 as Reported by a Nationwide Laboratory Service for 9.1 Million Tests[3]

SUBSTANCE	PERCENTAGE OF POSITIVE URINE DRUG SCREENS[a]
Marijuana	1.90
Amphetamines	0.90
Oxycodones	0.80
Benzodiazepines	0.71
Opiates	0.39
Cocaine	0.24
Barbiturates	0.22
Methadone	0.18
6-acetylmorphine (heroin)	0.03
Phencyclidine (PCP)	0.02

[a] Some samples had multiple drugs identified.

In 2014, 27.7 million persons (10.5% of Americans) reported driving under the influence of alcohol at least once during the past year with 10.2 million (3.9%) driving under the influence of illicit drugs and 30.9 million (11.7%) under the influence of alcohol and illicit drugs.[1] Of the 1.5 million people on parole and 4.4 million adults on probation in 2014, 23–29%, respectively, were current illicit drug users, and 16–15% were dependent or abusers of illicit drugs or alcohol.[1] In a 2013 study of 1681 male arrestees in five U.S. sites, there was a variable rate of congruence between self-reporting of drug use and positive urine drug screening (83% marijuana, 63% methamphetamine, 50% heroin, and 38% cocaine).[4]

TABLE 5-4. Categories of Substances Abused as Claimed by High School Seniors During 2014[5]

	PERCENTAGE OF SURVEY RESPONDENTS	
SUBSTANCE	PAST YEAR (%)	EVER (%)
Alcohol (to a drunken condition)	41.4	49.8
Any illicit drug	38.7	49.1
Marijuana and hashish	35.1	44.4
Amphetamines	8.1	12.1
Hallucinogens	4.0	11.4
Opioids (excluding heroin)	6.1	9.5
Tranquilizers	4.7	7.4
Sedatives	4.3	6.8
Inhalants	1.9	6.5
3,4-methylenedioxy-N-methamphetamine (MDMA, Ecstasy)	3.6	5.6
Cocaine	2.6	4.6
Methamphetamine	1.0	1.9
Androgenic anabolic steroids	1.5	1.9
Heroin	0.6	1.0

For eighth-grade, tenth-grade, and twelfth-grade students, the lifetime prevalence of the use of illicit drugs during 2014 was 20%, 37%, and 49%, respectively (**Table 5-4**).[5] During 2014, 51,966 people died from poisoning or overdose with 88 deaths (0.2%) occurring in children under five years of age.[6] Poisoning became the leading cause of injury-related death in the United States in 2008, and 91% of these deaths in 2014 were caused by drugs. The rate of drug-related, age-adjusted poisoning deaths has increased from 6.2 per 100,000 ($n = 17{,}415$) in 2000 to 14.6 per 100,000 ($n = 47{,}055$) in 2014—a 137% increase in 15 years.[6,7]

There is no comprehensive tabulation of all incidents of substance abuse or poisoning, and the available databases have strengths and weaknesses.[8,9] Nevertheless, substance abuse and poisoning are common problems facing healthcare professionals, law enforcement officials, employers, teachers, family members, and individuals throughout society. The detection and management of these incidents often involves laboratory testing and interpretation of the results. On a personal basis, healthcare professionals are asked by family members, acquaintances, and patients about drug testing and the potential impact on their lives. It is often prudent to refer the person to the testing laboratory or physician who ordered the test in question when pertinent facts are not available or when they are unable to be properly assessed. This chapter will focus on urine drug testing and serum drug concentration determinations as a means to aid in the management of substance abuse and poisoning.

URINE DRUG SCREENS

Objectives of Analysis

A *drug screen* provides a qualitative result based on the presence of a specific substance or group of substances. This determination is also called a *toxicology screen* or *tox screen.* Urine is the specimen of choice, and it is widely used for most situations requiring a drug screen. The collection of urine is generally noninvasive and can be collected following urinary catheterization in unresponsive patients. Adequate urine samples of 20–100 mL are easily collected. Most drugs and their metabolites are excreted and concentrated in urine. They are also stable in frozen urine allowing long-term storage for batched analyses or reanalysis. Urine is a relatively clean matrix for analysis due to the usual absence of protein and cellular components, thereby eliminating preparatory steps for analysis.[10-13]

A urine drug screen result does not provide an exact determination of how much of the substance is present in the urine. The concentration of the substance is actually measured by urine drug screen assays in the process of determining whether the drug is present in a significant amount to render the test as positive. For each substance, the test has performance standards established by the intrinsic specificity and sensitivity of the analytical process that are linked to regulatory or clinical thresholds, commonly called *cutoff values.* These thresholds are a balance of the actual analytical performance, likelihood for interfering substances, and the potential for false positives, which together suggest that the substance is actually present in the urine. Cutoff values may be set by an individual laboratory to meet regulatory or clinical needs, or by purchasing immunoassay kits with the desired cutoff values.

Regulatory cutoff values are typically used to monitor people in the workplace or patients undergoing substance abuse therapy. The Substance Abuse and Mental Health Services Administration (SAMHSA) in the Department of Health and Human Services specifies cutoff values for the drug categories that should be routinely included in urine screens for federal requirements (**Table 5-5**).[14] On October 1, 2017, SAMHSA will finalize the addition of MDA (3,4 methylenedioxyamphetamine, an amphetamine-like substance) and hydrocodone, hydromorphone, oxycodone, and oxymorphone (opioid drugs). (See the SAMHSA website for updated rules at www.samhsa.gov/workplace/drug-testing.)[14,15] In hospital and forensic settings, cutoff values are sometimes lowered relative to workplace values to detect more positive results, which can serve as an aid in verifying or detecting an overdose or poisoning.[13,16] Reports of urine drug screen results typically list the cutoff value for a substance and whether the substance was detected at the specified value.

TABLE 5-5. Federal Cutoff Concentrations for Urine Drug Tests[14,a]

DRUG	INITIAL TEST (ng/mL)	CONFIRMATORY TEST (ng/mL)
Amphetamine/ methamphetamine	500	250
MDMA/MDA	500	250
Cocaine metabolite[b]	150	100
Marijuana metabolite[c]	50	15
Codeine/morphine	2000	2000
Hydrocodone/hydromorphone	300	100
Oxycodone/oxymorphone	100	100
6-acetylmorphine[d]	10	10
PCP	25	25

PCP = phencyclidine; MDA = 3,4-methylenedioxyamphetamine; MDMA = 3,4-methylenedioxy-N-methamphetamine.

[a] Standards issued by Substance Abuse Mental Health Services Administration for urine specimens collected by federal agencies and by employers regulated by the Department of Transportation effective October 2017; website for changes (https://www.samhsa.gov/workplace/drug-testing).

[b] Metabolite as benzoylecgonine.

[c] Metabolite as delta-9-tetrahydrocannabinol-9-carboxylic acid.

[d] A metabolite specific to heroin.

General Analytical Techniques

There is no standardized urine drug screen that employs the same panel of tested drugs, analytical techniques, or turnaround times. Although there is some commonality among laboratories, tests differ by individual laboratory. Generally, urine drug screens are categorized by level of sensitivity and specificity of the analytical technique (preliminary versus confirmatory) and by the variety of drugs tested.[11,16] *Preliminary tests,* also known as *initial, provisional,* or *stat urine drug screens,* typically employ one of six currently available immunoassays (EMIT, KIMS, CEDIA, RIA, FPIA, or ELISA). (See Chapter 2.)

Immunoassays can be performed on autoanalyzers that are available in most hospitals. These assays are available for many substances of abuse, and results can be reported within one to two hours.[13,16] Many point-of-care tests (POCTs) also use an immunoassay technique, and results can be available within 5–15 minutes. Unfortunately, the result of an immunoassay is preliminary due to compromises in specificity that lead to cross-reactivity, particularly with amphetamines and opiates. A preliminary urine drug screen result cannot stand alone for medicolegal purposes and must be confirmed with another type of analysis that is more specific.[10,11] For clinical purposes, some laboratories routinely confirm the results of preliminary drug screens, but others do so only on request of the physician. The need for confirming preliminary test results is based on several factors: whether the result would affect the patient's care; whether the patient is expected to be discharged by the time the results are known; whether any legal actions are anticipated; and whether the cost justifies the possible outcome.

Confirmatory techniques are more specific than preliminary tests and utilize another analytical technique.[11,16] These tests include high-performance liquid chromatography (HPLC), gas chromatography, or mass spectrometry, depending on the substances being confirmed. The "gold standard" of confirmatory tests is the combination of *gas chromatography* and *mass spectrometry*, often referred to as *GC mass spec* or *GC-MS*. Compared to preliminary tests, these techniques are more time-consuming, more costly, require greater technical expertise, and require greater time for analysis—often several hours to days. Most hospital clinical laboratories do not have the capability to perform confirmatory tests and must send the specimen to a local reference laboratory or a regional laboratory. Transportation of the specimen will add to the delay in obtaining results. Confirmatory tests are routinely performed for workplace settings and forensic and medicolegal purposes, and the delay is often less critical than in clinical settings (**Minicase 1**).[10]

Common Applications

The purpose of a urine drug screen depends on the circumstances for its use, the condition of the patient, and the setting of the test. In an ED where a patient is being evaluated for a poisoning or overdose, the primary purposes are to verify substances claimed to be taken by the patient and to identify other toxins that could be likely causes of the poisoning or symptoms.[13] This is particularly important when the patient has altered mental status and cannot give a clear history or is experiencing nondrug causes of coma, such as traumatic head injury or stroke. The value of routinely performing urine drug screens in the ED for patients who overdose has been questioned.[17] The benefits include having objective evidence of the toxin's presence to confirm the exposure; suggesting alternative toxins in the diagnosis; ruling out a toxin as a cause of symptoms of unknown etiology; and providing medicolegal documentation. The disadvantages include being misled by false-positive results; impractical delays in receiving the results that do not influence therapy; and limited practical value because many poisonings can be recognized by a collection of signs and symptoms.[18]

MINICASE 1

Reliability of Amphetamine Results

Kisha T., a 21-year-old college student, is brought to the emergency department (ED) by her family because of bizarre behavior. She is having visual hallucinations and is paranoid and jittery. She is clinically dehydrated, tachycardic, and delirious. A stat preliminary urine drug screen is positive for amphetamines.

QUESTION: Is this patient abusing amphetamine?

DISCUSSION: Amphetamine abuse is possible, but alternative causes should be considered. Her parents report that she has just completed a week of final exams, is taking a full course load, and is working two part-time jobs. She is described as studious and a compulsive achiever. After six hours of supportive therapy, rest, and IV fluids, she is lucid and confesses to drinking more than a dozen energy drinks to stay awake in the past two days and taking two loratadine/pseudoephedrine 12-hour tablets six hours ago for allergy symptoms. A targeted confirmatory assay for amphetamines was negative for amphetamines and methamphetamine. Urine drug screens by immunoassay for amphetamines are subject to cross-reactivity with several sympathomimetic amine-type drugs (e.g., ephedrine and pseudoephedrine and their variants are often found in dietary supplements marketed for energy and weight loss and as decongestants), which would cause a false positive for amphetamines by immunoassay. Caffeine found in many energy drinks and dietary supplements for weight loss and energy is likely the principal cause of her symptoms. Caffeine was not detected in the urine screen because it was not on the testing panel of screened drugs.

The inclusion of a substance on a drug screen is also subject to individual laboratory discretion. Workplace and substance abuse monitoring programs are required to test for five categories of substances (marijuana metabolites, cocaine metabolites, opiate metabolites, phencyclidine [PCP], and amphetamines) as specified by the "Fed 5." Most immunoassay manufacturers design the range of assays to meet this need and offer additional categories that a laboratory may choose to include.[11,13] The expense of developing an immunoassay is balanced with the promise of economic recovery with widespread utilization. This economic reality precludes the development of a test for emerging substances of abuse, such as ketamine and γ-hydroxybutyrate, and life-threatening—albeit infrequent—overdoses, such as calcium channel antagonists and β-adrenergic blockers.[16] Techniques used for confirmatory tests would be required to detect many of the substances not included in the panel of the preliminary drug screen.

The American College of Emergency Physicians states in a clinical policy on the immediate treatment of poisonings that "qualitative toxicologic screening tests rarely assist the emergency physician in patient management."[19] Urine drug testing can be important with substances exhibiting delayed onset of toxic symptoms, such as sustained-release products, when patients ingest multiple agents, or when patients are found with multiple agents at the scene. Some trauma centers routinely perform urine drug screens on newly admitted patients, although the value of this practice has been questioned.[20] Suicidal and substance-abusing patients are poor or misleading historians, whereby the amounts, number of substances, and routes of exposure can be exaggerated or downplayed. A urine drug screen may assist in identifying potential substances involved in these cases and lead to specific monitoring or treatment (**Minicase 2**).

In the workplace, the purpose of a urine drug screen may include pre-employment tests, monitoring during work, post-accident evaluation, and substance abuse treatment monitoring.[10] Employers who conduct pre-employment urine drug tests will generally make hiring contingent on a negative test result. Many positions in the healthcare industry require pre-employment drug tests, and some employers perform random tests for employees in positions requiring safety or security as a means to deter drug use and abuse that could affect performance. In addition to random tests, some employers test individuals based on a reasonable suspicion of substance abuse such as evidence of use or possession, unusual or erratic behavior, or arrests for drug-related crimes. For employees involved in a serious accident, employers may test for substances when there is suspicion of use—to determine whether substance abuse was a factor—or as a necessity for legal or insurance purposes. Employees who return to work following treatment for substance abuse are often randomly tested as one of their conditions for continued employment or licensure. In the workplace setting, specific procedures must be followed to ensure that the rights of employees and employers are observed.

The Division of Workplace Programs of SAMHSA specifies guidelines for procedures, due process and the appeals process, and lists certified laboratories.[10] Two critical elements of workplace drug testing include establishing a chain-of-custody and control for the specimen and involving a medical review officer (MRO) to interpret positive test results. The chain-of-custody starts with close observation of urine collection. Patients are required to empty their pockets, and they are placed in a collection room without running water and where blue dye has been

MINICASE 2

Drug Screens and Emergent Care

Bob C., a 26-year-old male, is dropped off at an ED in the late evening after he became progressively more unresponsive in a hotel room. His acquaintances do not know his medical history but eventually admit that he had swallowed some drugs. They promptly leave the area. Bob C. is unconscious with some response to painful stimuli and exhibits pinpoint pupils and depressed respirations at 12 breaths/min. His other vital signs are satisfactory. Oxygen administration and intravenous (IV) fluids are started. A bedside stat glucose determination yields a result of 60 mg/dL. A 50-mL IV bolus of dextrose 50% is administered with no change in his level of consciousness. Naloxone 0.8 mg is given IV push, and within minutes Bob C. awakens, begins talking, and exhibits an improved respiratory rate. He admits to drinking some whiskey and taking a handful of several combination tablets of hydrocodone and acetaminophen shortly before he was dropped off at the ED.

In addition to routine laboratory assessment, a serum acetaminophen concentration is determined. During the next 24 hours, he receives supportive care in the critical care unit and requires two additional doses of naloxone. He is scheduled for a psychiatric evaluation to assess treatment options for his substance abuse, but he walks out of the hospital against medical advice on the second day. A urine drug screen by immunoassay that was obtained in the ED is reported as positive for opiates and marijuana on the morning of his second day of hospitalization. The serum acetaminophen concentration reported two hours after ED arrival was 60 mcg/mL, which was obtained approximately six hours after drug ingestion. Ethanol was not included in the drug screen panel.

QUESTION: Was a urine drug screen necessary for the immediate care of this patient? How is a urine drug screen helpful in this type of situation?

DISCUSSION: In emergent situations like this one that involve an apparent acute opiate overdose, the results of a urine drug screen are not necessary for immediate evaluation and effective treatment. The symptoms and history clearly indicate that an opiate overdose is very likely.[21] The response to naloxone confirms that an opiate is responsible for the central nervous system (CNS) depressant effects. Because immediate treatment was necessary, waiting for the results of the preliminary drug screen—even if it was reported within hours—would not change the use of supportive care, glucose, and naloxone. The urine drug screen may be helpful to confirm the diagnosis for the record and to assist in guiding substance abuse treatment. Obtaining a serum acetaminophen concentration is important in cases of intentional drug use (i.e., suicide attempt and substance abuse). This practice is particularly important in situations of a multiple drug exposure, an unknown drug exposure, or when acetaminophen may be contained in a multiple-ingredient, oral drug product (e.g., analgesics, cough and cold medicines, sleep aids, and nonprescription allergy medicines). A serum specimen is needed because acetaminophen is not part of routine urine drug screens, and serum assays on acetaminophen generally have a quick turnaround time so they can be used clinically to assess the potential severity of the exposure. In this patient's case, the serum acetaminophen concentration did not indicate a risk for hepatotoxicity. Another benefit of obtaining a serum acetaminophen concentration in this case is that it indirectly confirms that an opioid combination product was involved and is consistent with his response to naloxone.

added to the toilet water. These measures minimize the risk of adulteration or dilution of the urine sample. After the urine is placed in the container, the temperature is taken, the container is sealed, and the chain-of-custody documentation is completed. After the chain-of-custody form is completed by everyone in possession of the specimen, it reaches the laboratory where the seal is broken and further procedures are observed. Positive specimens are often frozen for one year or longer if requested by the client or if the results are contested by a court. Chain-of-custody procedures are time-consuming and are not typically considered in the clinical management of poisonings and overdoses, but they are important to sustain the validity of the sample and its result in a court of law.[10]

An MRO is typically a physician trained in this specialty who has responsibility to determine whether the result of the drug test is related to substance abuse.[10,22] Duties involve interviewing the donor; reviewing his or her therapeutic drug regimen; reviewing possible extraneous causes of a positive result, such as a false-positive result from a prescribed medication or substance interfering with the analytical test; rendering an opinion on the validity of the test result; considering a retest of the donor or the same specimen; reporting the result to the employer; and maintaining confidential records. This individualized interpretation is not only critical because people's careers, reputations, livelihood, and legal status can be affected, but also because it is a regulatory requirement.

Drug screening is also used in the criminal justice system for several purposes such as informing judges for setting bail and sentencing, monitoring whether specified drug abstinence is being observed, and identifying individuals in need of treatment.[4] For example, a positive drug test at the time of arrest may identify substance abusers who need medical treatment prior to incarceration, which may result in a pretrial release condition that incorporates periodic drug testing. If a defendant is being monitored while on parole or work release, a drug screen can verify that he or she is remaining drug free. Drug tests in prisons can also assist in monitoring substance use in jail.

The impact of a drug screen result can be profound if it affects decisions of medical care, employment, legal importance, and a person's reputation. In addition, several factors can affect the reliability and interpretation of drug screen results. These issues should be considered when evaluating a drug screen and are described in the following section.

Unique Considerations

When a urine drug screen is reported as negative, it does not mean that the drug was not present or not taken—it means that it was not detected. The drug in question may not be part of a testing panel of the particular drug screen (**Table 5-6**). For example, meperidine and fentanyl are not detected on opiate immunoassays.[13,16] Illicitly synthesized and manufactured analogs or homologs of drugs or substances, also called *designer drugs,* are not detected by routine drug screens because the chemical structure is often unknown, a reference standard and assay have yet to be developed, and variations of a chemical structure are frequently introduced into the illicit drug marketplace. Another factor may involve urine that is too dilute for detection of the substance. This may be due to renal disease, intentional dilution to avoid detection, or administration of large volumes of IV fluids as part of a critically ill patient's care. The urine may have been collected before the drug was excreted, but this is unlikely in symptomatic acute overdoses or poisonings. The time that an individual tests positive (i.e., the drug detection time) depends on pharmacologic factors including dose, route of administration, rates of metabolism and elimination, and analytical factors (e.g., sensitivity, specificity, and accuracy). In some cases, the urine sample may have been intentionally adulterated to mask or avoid detection.

Adulteration of a urine sample either intentionally or unintentionally can lead to negative or false-positive results through several means.[10,23,24] A freshly voided urine sample may be replaced with a drug-free sample when urine collection is not directly observed. The ingestion of large volumes of water with or without a diuretic may dilute a drug in the urine, thereby reducing the concentration of the urine below the assay detection limit. Urine specimens for workplace testing and substance abuse monitoring will be tested for temperature within four minutes of collection and later tested for creatinine concentration, pH, specific gravity and the presence of oxidizing adulterants

TABLE 5-6. Categories of Drugs and Chemicals Often Not Detected by Routine Drug Screens[a]

Androgenic anabolic steroids
Anesthetics (e.g., ketamine, lidocaine)
Angiotensin-converting enzyme inhibitors
Animal venoms
Antidysrhythmic drugs
Anticoagulant drugs
β-adrenergic agonists
β-adrenergic antagonists
Calcium channel antagonists
Chemical terrorism agents
Dietary supplements[b]
Designer cannabinoids
Designer cathinones (e.g., bath salts, flakka)
Designer phenethylamines (e.g., 2Cs, N-BOMe drugs)
Heavy metals (e.g., lead, arsenic, and mercury)[c]
Hydrocarbon solvents and inhalants
Nonbenzodiazepine hypnotics (e.g., zolpidem, eszopiclone, zaleplon)
Pesticides
Plant toxins
Selective serotonin reuptake inhibitors
Synthetic opioids (e.g., fentanyl, meperidine, methadone)

[a] See Quickviews for more examples

[b] Those without chemically similar drug counterparts are not detected on a drug screen.

[c] Heavy metals will require a special collection container, collection duration, and assay.

(e.g., chromates, nitrites) as part of routine specimen validity testing.

Adding a chemical to a urine sample may invalidate some test results. Adulteration products that are available through the Internet contain chemicals such as soaps, glutaraldehyde, nitrites, other oxidants, and hydrochloric acid. Depending on the assay method and test, these substances may interfere with absorbance rates or enzyme activity, produce false-positive or false-negative results, or oxidize metabolites that are measured in the immunoassay. For example, some chromate-based and peroxidase-based oxidizers will degrade 9-carboxy-tetrahydrocannabinol, a principal metabolite of tetrahydrocannabinol, and lead to a negative result for marijuana.[23,24] Taking large amounts of sustained-release niacin (2.5–5.5 g over 36–48 hours) has been promoted on the Internet as a means to rid the body of cocaine and marijuana and interfere with urine drug screens. This practice is unlikely to produce the desired outcome, but it has produced niacin poisonings ranging from skin flushing to life-threatening symptoms that required hospitalization.[25] The effects of adulterants vary with the immunoassay technique and the specific test used by the laboratory; they are not reliable ways to mask drug use. Most adulterants do not affect the GC-MS analysis for drugs in urine, but such a confirmatory step would be ordered only if there was a high suspicion of adulteration. A positive immunoassay result is typically used to justify the use of a confirmatory GC-MS analysis.

A positive drug test can show the presence of specific drugs in urine at the detectable level of the test. It does not indicate the dosage, when the drug was administered, how it was administered, or the degree of impairment. Many drugs can be detected in urine for up to three days after being taken and some up to two weeks or more (**Table 5-7**).[11,13,24] It is possible for a legitimate substance in the urine to interact with the immunoassay and produce a false-positive result.[13,24,26,27]

Exposure to interfering substances can affect the results of an immunoassay urine drug screen (Table 5-7). A positive immunoassay result for opiates may result from the ingestion of pastries containing poppy seeds because they contain codeine and morphine in small, but sufficient, amounts to render the test positive. The result is a true positive but not a positive indicator of drug abuse. The immunoassay for amphetamines is prone to

TABLE 5-7. Detection Times and Interfering Substances for Immunoassay Urine Drug Screens[a,b,c]

DRUG	DETECTION TIME	POTENTIAL FALSE-POSITIVE AGENTS AND COMMENTS
Amphetamines	2–5 days; up to 2 weeks with prolonged or heavy use	Ephedrine, pseudoephedrine, ephedra (ma huang), phenylephrine, selegiline, chlorpromazine, promethazine, trazodone, bupropion, desipramine, trimipramine, ritodrine, amantadine, ranitidine, phenylpropanolamine, brompheniramine, 3,4-methylenedioxy-N-methamphetamine (MDMA, Ecstacy), isometheptene, labetalol, phentermine, methylphenidate, isoxsuprine, trimethobenzamide
Barbiturates	Short-acting, 1–7 days; intermediate-acting, one to 3 weeks	Ibuprofen, naproxen; phenobarbital may be detected up to 4 weeks
Benzodiazepines	Up to 2 weeks; up to 6 weeks with chronic use of some agents	Oxaprozin, sertraline; benzodiazepines vary in cross-reactivity, persistence, and detectability; flunitrazepam may not be detected
Cocaine metabolite (benzoylecgonine)	12–72 hr; up to 1–3 weeks with prolonged or heavy use	Cross-reactivity with cocaethylene varies with the assay because assay is directed to benzoylecgonine; false positives from -caine anesthetics and other drugs are unlikely
Lysergic acid diethylamide (LSD)	1–2 days typically; up to 5 days possible	
Marijuana metabolite (delta 9-tetrahydrocannabinol-9-carboxylic acid)	7–10 days; 1 month or more with prolonged or heavy use	Ibuprofen, naproxen, tolmetin, efavirenz, pantoprazole; patients taking dronabinol will also have positive test results
Methadone	3–14 days	Diphenhydramine, doxylamine, clomipramine, chlorpromazine, thioridazine, quetiapine, verapamil
Opioids	2–3 days typically; up to 6 days with sustained-release formulations; up to 1 week with prolonged or heavy use	Rifampin, some fluoroquinolones, poppy seeds, quinine in tonic water; the assay is directed toward morphine with varying cross-reactivity for codeine, oxycodone, hydrocodone, and other semisynthetic opioids; synthetic opioids (e.g., fentanyl, meperidine, methadone, pentazocine, propoxyphene, and tramadol) have minimal cross-reactivity and may not be detected
Phencyclidine (PCP)	2–10 days; 1 month or more with prolonged or heavy use	Ketamine, dextromethorphan, diphenhydramine, imipramine, mesoridazine, thioridazine, venlafaxine, ibuprofen, meperidine, tramadol

[a] Time after which a drug screen remains positive after last use.

[b] Because performance characteristics may vary with the type of immunoassay, manufacturer, and lot, consult the laboratory technician and package insert for the particular test.

[c] Data from references 11, 24, 26, and 27.

false-positive results from drugs with similar structures such as ephedrine, pseudoephedrine, and buproprion.[13,26] Also, drugs seemingly dissimilar from the target of an immunoassay can cause false-positive results. For example, naproxen can produce false-positive results for marijuana and barbiturates and was found to do so in 1 of 14 volunteers tested.[28] Most fluoroquinolone antibiotics can produce false-positive opiate results, but this interference varies with the fluoroquinolone and immunoassay.[29] The immunoassay manufacturer's package insert should be consulted for information on known interfering substances. In workplace settings, the MRO is obligated to assess whether a person's legitimate drug therapy could interfere with the result (**Minicase 3**).

The persistence of the substance in the urine is an important factor in the interpretation of the results (Table 5-7).[26] For laboratory results reported as negative, it may indicate that the specimen was obtained too early or too late after exposure to a chemical, thereby producing a urine specimen with insufficient concentration of the drug to lead to a positive result. Drugs with short half-lives, such as amphetamines, may not be detectable several hours after use. A common concern for individuals undergoing workplace testing is the length of time after use that the drug will still be detectable. This will vary with the sensitivity of the assay; whether the assay is directed to the parent drug or the metabolite; whether the drug or its metabolites exhibits extensive distribution to tissues that will affect its half-life; the dose of the drug taken; and whether the drug was used chronically or only once. For example, cocaine is rarely detected in a urine specimen because of its rapid metabolism. Immunoassays are directed to cocaine metabolites, such as benzoylecgonine, which are detected for up to two to three days after use and up to eight days with heavy use. The major active component of marijuana, delta-9-tetrahydrocannabinol, is converted to several metabolites of which delta-9-tetrahydrocannabinol-9-carboxylic acid is the agent to which antibodies are directed in many immunoassays. This metabolite is distributed to tissues and can be detected for days to weeks after use.[13,26] Chronic or heavy use can lead to detection up to a month or more after stopping use (**Minicase 4**). Immunoassays may lead to false-negative reports in part due to incomplete cross-reactivity across a drug class. For example, the benzodiazepine immunoassay is designed to detect oxazepam, nordiazepam, and temazepam, which are metabolites of diazepam, but the assay does not react with alprazolam, clonazepam, or lorazepam.[30]

For clinical applications, the time it takes for the test result to be reported to the clinician after specimen collection, also known as *turnaround time*, can affect the utility of the drug screen. Many hospital laboratories can perform preliminary immunoassay urine drug screens using mechanized analytical technology, which is used for common clinical tests or using dedicated desktop analyzers. Results from in-hospital laboratories can often be returned within two hours of collection. For many urgent situations, such as an acute overdose or poisoning, this delay is unlikely to influence the immediate therapy of the victim. The results may lead to later consideration of alternative or additional diagnoses. Most clinics, small hospitals, or specimen collection sites do not possess such capability and must rely on making the specimen a *send out* that is performed at a nearby or regional reference laboratory. The turnaround time from a reference laboratory varies with the laboratory and the need for urgency. Most results for clinical applications are reported within 24–48 hours. However, some results may take up to three to seven days. In some situations, such as pre-employment workplace testing, this delay is acceptable. The turnaround time for confirmatory testing depends on

MINICASE 3

Workplace Drug Screen Interpretations

Juan G., a 45-year-old pharmacist, applies for a position at a hospital pharmacy. As part of his pre-employment evaluation, he is asked to provide a urine specimen in a specially designed room for drug testing. His urine sample is positive for opiates and marijuana by immunoassay. His case is referred to the hospital's MRO for a review of the findings. The physician orders a confirmatory test on the same urine specimen. The human resources department of the hospital learns from his current employer that he is an above average worker with no history of substance abuse. A criminal background check is negative for any criminal record. The MRO contacts Juan G. and learns that he was taking acetaminophen and codeine prescribed for pain from suturing of a laceration of his hand for two days prior to drug testing. He also routinely takes naproxen for arthritis in his knees. He had forgotten to list the recent use of these drugs on his employment application because his injured hand began to ache while writing.

QUESTION: Has this patient used any drugs or substances that should prevent him from being considered for employment?

DISCUSSION: Consideration of several factors is important in interpreting the urine drug screen result in this case. The patient has no obvious symptoms of intoxication and has a good employment record. It is likely that the codeine prescribed for pain control produced the positive opiate result. This drug is being used for a legitimate purpose with a valid prescription. The positive test for marijuana is likely from his use of naproxen causing a false-positive result. The confirmatory test by GC-MS was negative for marijuana, but it was positive for codeine and morphine. Codeine is metabolized in part to morphine. The MRO reviewing this case would likely conclude that the test results are not indicative of opioid abuse and the marijuana immunoassay result was a false positive. If there are concerns about his suitability for employment, he may be subjected to an unannounced drug test during his probationary employment period. Acetaminophen and naproxen were not reported as a result, because they were not on the routine assay panel.

MINICASE 4

Evidence of Heroin

Danny W., a 23-year-old assembly line worker at a computer manufacturing facility, is examined by the company's physician within an hour of being involved in a workplace accident. She observes a laceration on his left arm, pupil size of 1–2 mm (normal, 2–5 mm), bilateral ptosis, and recent punctate lesions on the left antecubital fossa. The rest of the physical exam is unremarkable. Danny W. denies eating poppy seeds, taking any medication or dietary supplement, or having a neurological condition. He has no history of substance abuse in his files. The physician suspects heroin use and orders a focused urine drug test for opiates. Several days later, the laboratory report indicates positive results for morphine, codeine, and 6-acetylmorphine.

QUESTION: Has this patient used a drug or substance that would impair his ability to work? What, if any, substance is likely?

DISCUSSION: This patient has likely used heroin several hours before the accident and several symptoms are consistent with opiate intoxication. Heroin may not be present in sufficient amounts to be detected, in part, because it is metabolized to several compounds such as morphine and 6-acetylmorphine, which can be detected in the urine of heroin users. Because 6-acetylmorphine is only found in urine following heroin use, its presence confirms heroin but other opioids could also contribute to his symptoms. The presence of small amounts of codeine in heroin abusers is likely from contamination of the heroin with codeine and is not because codeine is a metabolic byproduct of heroin, or because he had consumed codeine.

MINICASE 5

Interpreting Cocaine Results

Shelly N., a 56-year-old supervisor for a large utility company, had recently conducted an inspection at a nuclear power plant. She then left for a two-week vacation with a friend. After returning to work, she is asked to submit a urine sample for drug testing because the company performs random drug tests for compliance with regulatory, insurance, and contractual requirements. A week later, the results of the immunoassay are reported as positive for the cocaine metabolite, benzoylecgonine. A confirmatory test by GC-MS confirms the immunoassay result. Shelly N. is asked to report to the company's medical office. During the interview with the physician, she denies illicit drug use but states that she had dental work performed immediately before returning to work from her vacation and that she had received procaine hydrochloride (Novocain) for local anesthesia.

QUESTION: What caused the positive test result for cocaine?

DISCUSSION: This patient apparently believed that any substance with a name ending in *-caine* must share chemical similarity with cocaine and could be a probable cause of a false-positive result. Although interference with immunoassays is possible, a false positive for cocaine with local anesthetics is not likely unless the anesthetic preparation contains cocaine. The positive result was confirmed by a confirmatory test that is not subject to this type of interference. In this patient's case, use of cocaine is the most likely explanation for the positive result.

the laboratory, transportation time from the collection site to the laboratory, the tests being performed or requested, and the need for urgency. The delay could be as short as 24 hours or as long as a month or more, particularly for postmortem samples (**Minicase 5**).

SERUM CONCENTRATIONS

Objectives of Analysis

Quantitative assays determine the concentration of a substance in a biological specimen, typically this involves serum. The availability of serum concentrations for toxins is based on considerations of whether the concentration correlates with an effect; the outcome or need for therapy; the existing use of the assay for another application such as therapeutic drug monitoring; and technical ease of performing the assay. Serum is typically not used for drug screening purposes in clinical or workplace settings.

Many poisonings and overdoses can be adequately managed without quantitative analysis.[9] A history of the exposure, signs and symptoms, and routinely available clinical tests—such as full blood count, electrolytes, glucose, international normalized ratio (INR), liver function tests, blood urea nitrogen, serum creatinine, anion gap, serum osmolality and osmolal gap, arterial blood gases, and creatinine kinase—can guide patient management decisions. Intravenous lipid emulsion (ILE) therapy (e.g., infusing Intralipid) is an increasingly used rescue therapy for toxicity and poisoning from local anesthetics and highly lipophilic drugs. There is mounting evidence that the resulting lipemia from ILE may affect common clinical laboratory tests and drug concentration assays leading to spurious results

TABLE 5-8. Examples of the Use of Therapies to Treat Toxicity Guided by Serum Concentrations

THERAPY	DRUG OR TOXIN
Antidote	Acetaminophen, ethylene glycol, methanol
Chelation	Iron, lead
Hemodialysis	Ethylene glycol, lithium, methanol, salicylate, theophylline, valproic acid
Multiple-dose activated charcoal	Carbamazepine, phenobarbital, theophylline, valproic acid
Toxin-specific antibody	Digoxin
Urine alkalinization	Phenobarbital, salicylate

when the blood sample is drawn during or close to the administration of ILE.[31]

Serum concentrations of potential toxins can be complementary to clinical tests or become essential in several situations (**Table 5-8**).[16] A serum concentration can confirm the diagnosis of a poisoning when in doubt or when a quantitative assessment in the serum is important to interpret a qualitative urine drug screen. When there is a relationship between serum concentration and toxicity, a serum concentration can assist in patient evaluation or for medicolegal purposes. When sustained-release drug formulations have been ingested, serial serum concentrations can indicate when peak serum concentrations have occurred and whether efforts to decontaminate the gastrointestinal tract with activated charcoal or whole bowel irrigation have been achieved. A serum concentration can also be useful in determining when to reinitiate drug therapy after the drug has caused toxicity. For some agents, serum concentrations can guide the decision to use therapies that are often risky, invasive, or expensive such as antidotes (e.g., acetylcysteine, digoxin immune antibody, and fomepizole) or special treatments (e.g., hemodialysis and hyperbaric oxygen).

General Analytical Techniques

There is no standardized panel of quantitative serum assays for toxicologic use, and the availability of tests differs by individual laboratory. Generally, serum concentrations utilize existing technologies (e.g., immunoassay, spectrophotometry, gas chromatography, HPLC, and atomic absorption spectrometry) that are commonly used for therapeutic drug monitoring (see Chapter 2). Assays for carboxyhemoglobin, methemoglobinemia, and serum cholinesterase activity are available in many hospitals.[16] In most toxicological applications, the specimen is usually 5–10 mL of blood in adults (1–5 mL in children depending on the assay) that has been allowed to clot for several minutes. It is then centrifuged and the clear serum, which is devoid of red blood cells and coagulants, is aspirated and subjected to analysis or frozen for later analysis. The type of test tube, test tube additive, and quantity of blood necessary should be verified with the laboratory prior to blood collection.

Common Applications

Serum concentrations of several drugs and chemicals can be helpful in the assessment of patients who may be poisoned or overdosed and arrive at a hospital for evaluation and treatment. Although general treatment approaches—such as supportive care, resuscitation, symptomatic care, and decontamination—are performed without the need of serum concentrations, the severity of several toxicities are related to serum concentrations (Table 5-8). The examples of ethanol, salicylates, acetaminophen, and digoxin demonstrate important principles in the application of serum concentrations to toxicity and therapy.

One of the most widely studied and used toxicologic tests involves blood, serum, or breath ethanol concentrations. Because of the absence of protein binding and small volume of ethanol distribution, the serum concentration generally correlates with many of the acute toxic effects of ethanol as shown in **Table 5-9**.[32] Regular ethanol use can lead to tolerance, and ethanol concentrations in excess of 0.4% (400 mg/dL) can easily be tolerated by some patients (e.g., they can converse and exhibit stable vital signs).[33,34] Conversely, uninitiated drinkers, such as small children who ingest household products containing ethanol (e.g., cologne and mouthwash) and those who concurrently ingest other CNS depressants, may have an exaggerated effect. Most acute poisonings can be managed with supportive and symptomatic care; an unstable patient with exceedingly high ethanol concentrations may be the rare candidate for hemodialysis.

Ethanol concentrations also have medicolegal applications involving driving or work performance and ethanol intake. In 2005, the minimum legal threshold for driving under the influence of ethanol intoxication was set by all states of the United States at blood ethanol concentrations of 0.08% (equivalent to 0.08 g/dL or 80 mg/dL).[32] This value can be determined at the scene or at bedside by a breath alcohol test.[35] The breath alcohol test is based on the assumption that equilibrium

TABLE 5-9. Relationship of Blood Ethanol Concentration and Toxic Effects[32]

BLOOD ETHANOL CONCENTRATION	TOXIC EFFECT OR CONSEQUENCE
0.08% (80 mg/dL)	Legal definition for driving impairment
0.15% (150 mg/dL)	Euphoria, loss of critical judgment, slurred speech, incoordination, drowsiness
0.2% (200 mg/dL)	Increased incoordination, staggering gait, slurred speech, lethargy, disorientation, visual disturbances (diplopia, reduced acuity and perception)
0.3% (300 mg/dL)	Loss of motor functions, marked decreased response to stimuli, impaired consciousness, marked incoordination and inability to stand or walk, vomiting and incontinence, possible amnesia of the event
0.4% (400 mg/dL) and higher	Comatose, unresponsive to physical stimuli, absent reflexes, unstable vital signs, shallow and decreased respirations, hypotension, hypothermia, potentially lethal

exists between ethanol in the blood supply of the lung and the alveolar air at a relatively uniform partition ratio. A number of variables can affect this relationship such as temperature, hematocrit, and sampling technique. The National Highway Traffic and Safety Administration publishes a list of breath alcohol testing devices that conform to their standards (www.nhtsa.gov).

Because ethanol concentrations are reported in several different units for either serum or blood, verification of the unit of measure is important.[16,36] Further, many hospital-based laboratories perform ethanol determinations on serum and use the units of mg/dL versus forensic situations that typically use blood and report the value as %, g%, or g/dL (all of which are equivalent expressions except mg/dL). Serum concentrations of ethanol are greater than blood concentrations by a median factor of 1.2, which varies with the hematocrit value because of the greater water content of serum compared to whole blood.[13,16] Although legal standards are written in terms of whole blood concentrations, this difference is without clinical significance.

Ethanol is also used as a drug to treat poisonings by methanol (blindness, acidosis, and death) and ethylene glycol (acidosis, renal failure, and death). To achieve a consistent concentration near 100 mg/dL, serial serum ethanol concentrations are obtained to ensure that sufficient quantities have been administered to prevent severe toxicities of methanol and ethylene glycol (during therapy, hemodialysis removes ethanol while also removing methanol and ethylene glycol).

An early attempt to correlate serum drug concentrations with acute toxicity over time involved the Done nomogram for salicylate poisoning.[37] Categories of toxicity (mild, moderate, and severe) were demarcated on a semilogarithmic plot of serum salicylate versus time after ingestion as an aid to interpreting serum concentrations. Given the limited knowledge available at the time, the nomogram was based on several assumptions (zero-order kinetics and back extrapolation of single concentrations to time zero) that were later proven to be false. The nomogram did not guide therapy to any great extent and was not confirmed to be clinically useful in subsequent studies.[38]

Clinical findings such as vital signs, electrolytes, anion gap, and arterial blood gases, which have quick turnaround times in most hospitals, are more direct indicators of salicylate toxicity and are now preferred to the Done nomogram. A patient, with exceedingly high serum salicylate concentrations (in excess of 90–100 mg/dL) who is unresponsive to supportive and symptomatic therapy, may benefit from hemodialysis to remove salicylate from the body. Elderly and very young patients with unexplained changes in consciousness, acid–base balance, and respiratory rate who present to an ED could be suffering from unrecognized acute or chronic salicylate poisoning.[39,40] A routine serum salicylate concentration in such patients could determine the contribution of excessive salicylate to their symptoms.

Serum acetaminophen concentrations following acute overdoses are essential in assessing the potential severity of poisoning and determining the need for antidotal therapy with acetylcysteine.[41,42] Acetaminophen toxicity differs from many other poisonings in that there is delay of significant symptoms by one to three days after ingestion, whereas most other poisonings have definite symptoms within six hours of exposure.[9] This delay in onset makes it difficult to use signs, symptoms, and clinical diagnostic tests (such as serum transaminase, bilirubin, or INR) as an early means to assess the risk of acetaminophen toxicity.[9] A serum concentration of acetaminophen obtained at least four hours after an acute ingestion (**Figure 5-1**) can be used to assess whether a patient is at risk of developing acetaminophen hepatotoxicity.[41,42] The acetaminophen nomogram is intended to be used only for an acute, single-episode ingestion of immediate-release acetaminophen and not in situations when acetaminophen is ingested in supratherapeutic doses over several hours or days.

The semilogarithmic plot of serum acetaminophen concentration versus time, also called the *Rumack-Matthew nomogram* or *acetaminophen nomogram,* is also used to determine whether there is a need to administer acetylcysteine to reduce the risk of toxicity.[41,42] If the results are not expected to be available within 10 hours of ingestion, acetylcysteine is typically administered provisionally and then continued or discontinued based on the serum acetaminophen concentration. In situations when the specimen is sent to a reference laboratory, the results may take several days to be reported, and, consequently, the patient may receive the entire course of therapy that may last for 72 hours with the oral regimen or 21 hours with the IV regimen. Because of the widespread availability of acetaminophen, it is commonly ingested in suicide attempts. Several professional groups have advocated that all patients who are suspected of intentionally taking drugs should have a serum acetaminophen concentration determined as part of their evaluation in the ED.[19] In the case of acetaminophen poisoning,

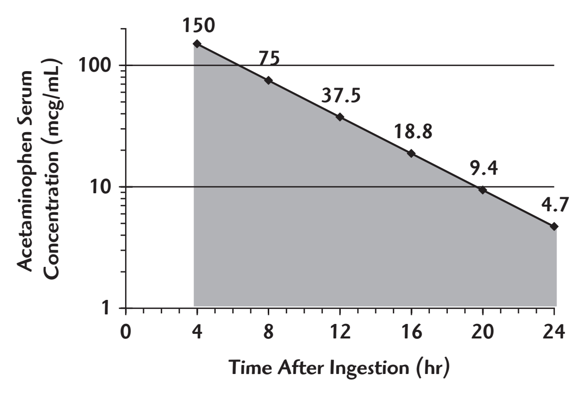

FIGURE 5-1. Risk of hepatotoxicity after an acute ingestion of acetaminophen. Acetaminophen serum concentrations in the shaded area are not associated with hepatotoxicity; whereas, those above the line are at risk for developing hepatotoxicity and acetylcysteine therapy should be considered.

MINICASE 6

Value of Acetaminophen Concentrations

A mother calls a poison control center about her 16-year-old daughter, Kelly A., who has acutely ingested approximately 30 acetaminophen 500-mg tablets one hour ago. She thinks that her daughter was "trying to hurt herself." The pharmacist at the poison center refers Kelly A. to the nearest hospital for evaluation due to the amount of acetaminophen and the intent of the ingestion. The mother is asked to bring any medicine to which Kelly A. may have had access. At the ED, Kelly A. vomits several times but has no other physical complaints or symptoms. A physical exam is unremarkable except for the vomiting. Baseline electrolytes, complete blood count, liver function tests, urine drug screen, and a pregnancy test are ordered. An IV line is placed and maintenance IV fluids are started. At four hours after the acetaminophen ingestion, a blood specimen is drawn to determine the serum acetaminophen concentration. Ninety minutes later, the result is reported as 234 mcg/mL.

QUESTION: Is this patient at risk for acetaminophen hepatotoxicity? Should she be treated with acetylcysteine?

DISCUSSION: When the serum acetaminophen concentration of 234 mcg/mL is plotted on the acetaminophen nomogram at four hours, it is clearly above the treatment line. This indicates that this patient is at risk for developing hepatotoxicity and that treatment with acetylcysteine should be initiated immediately.[41] The dose of acetaminophen that she ingested is also associated with a risk of developing hepatotoxicity, but patients with intentional overdoses (substance abuse or attempted suicide) do not always provide accurate histories. If the results of the acetaminophen assay would not have been available within 2 hours of sampling or within 8–10 hours of ingestion, acetylcysteine therapy would have been started provisionally. After learning the acetaminophen concentration, the physician would have decided to continue or stop acetylcysteine. Because most patients do not exhibit signs and symptoms of acute hepatic injury until one to three days after acute acetaminophen overdose, serum transaminase and bilirubin values would not be expected to be abnormal at the time of this patient's assessment in the ED.

the serum concentration becomes a valuable determinant of recognition, therapy, and disposition (**Minicase 6**).

A serum concentration can also guide the utilization or dosage determination of antidotes that are in short supply or are expensive, such as digoxin immune fragment antibody (DigiFab). Life-threatening acute or chronic digoxin toxicity may require the administration of digoxin immune fragment antibody to quickly reverse the toxic effects of digoxin. The dose of digoxin immune fragment antibody can be determined empirically, based on the amount ingested, or by a steady-state serum concentration (consult current prescribing information).[43,44]

Number of vials of digoxin immune fragment antibody
= serum concentration of digoxin (ng/mL)
× patient weight (kg)/100

Once the digoxin immune fragment antibody is administered, the serum concentration of digoxin precipitously rises and has no correlation to the degree of toxicity (**Figure 5-2**).[43,44] This sharp increase of digoxin reflects total digoxin (protein-bound and unbound) in the serum that has been redistributed from tissue sites. The digoxin bound to digoxin immune fragment antibody is not toxicologically active, and it is eventually excreted in the urine.

Unique Considerations

The timing of sample collection for poisoned or overdosed patients is variable due to the varying times of arrival at an ED after the exposure and the delay in the recognition of poisoning (unless it is obvious from the history or symptoms).[45] Most specimens are collected at the time of admission to the ED except when specified times are important, such as acetaminophen or when adequate absorption has yet to occur (Table 5-8). This variability makes comparison of serum concentrations among patients difficult to clearly establish a relationship with the concentration and toxicity.

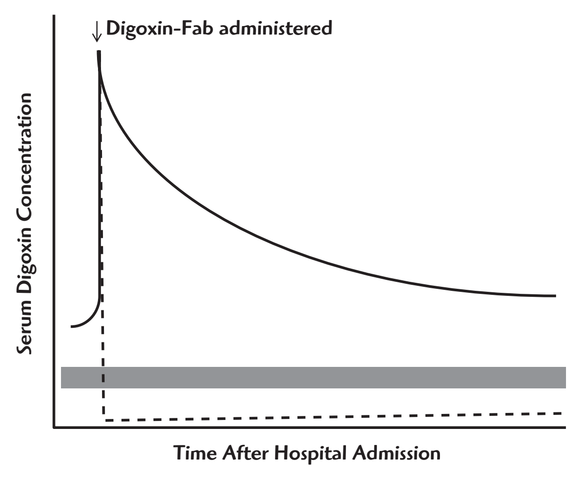

FIGURE 5-2. Simulated serum digoxin concentrations before and after administration of digoxin fragment antibodies (Fab). Total digoxin concentrations (solid line) rapidly rise to often dramatically high concentrations after administration of digoxin-Fab. Total digoxin concentrations fall with the excretion of digoxin bound to Fab. Free digoxin concentrations (dashed line) are associated with pharmacologic activity and rapidly drop to low or unmeasurable concentrations within one hour of digoxin-Fab administration. The shaded area represents the range of therapeutic serum digoxin concentrations.

TABLE 5-10. Influence of Toxicokinetic Changes in Poisoned and Overdosed Patients

EFFECT OF OVERDOSAGE[a]	EXAMPLES
Slowed absorption due to formation of poorly soluble semisolid tablet masses in the gastrointestinal tract	Aspirin, lithium, phenytoin, sustained-release theophylline
Slowed absorption due to slowed gastrointestinal motility	Benztropine, nortriptyline
Slowed absorption due to toxin-induced hypoperfusion	Procainamide
Decreased serum protein binding	Lidocaine, salicylates, valproic acid
Increased volume of distribution associated with toxin-induced acidemia	Salicylates
Slowed elimination due to saturation of biotransformation pathways	Ethanol, phenytoin, salicylates, theophylline
Slowed elimination due to toxin-induced hypothermia (<35 °C)	Ethanol, propranolol
Prolonged toxicity due to formation of longer-acting metabolites	Carbamazepine, dapsone, glutethimide, meperidine

[a] Compared to characteristics following therapeutic doses or resolution of toxicity.
Source: Reprinted, with permission, from reference 9.

The pharmacokinetics of drugs and chemicals on overdose, sometimes termed *toxicokinetics*, can affect interpretation of a serum concentration.[9] Few studies have compared the pharmacokinetics of drugs in therapeutic and toxic doses because toxic doses cannot be administered to human volunteers and overdosed patients are too heterogeneous to make clear assessments. Nevertheless, there are several examples where the absorption, distribution, metabolism, and elimination of drugs are different on overdose (**Table 5-10**). It is generally inappropriate to apply pharmacokinetic parameters derived from therapeutic doses to situations when massive overdoses are involved. Many patients who are poisoned or have overdosed are critically ill, and multiple samples of blood have been obtained for a variety of tests to monitor their condition. When the toxic agent is recognized late in the course of therapy or when serial determinations could be helpful in understanding some aspect of therapy or toxicity, scavenging aliquots of existing serum samples may be helpful for retrospective toxicological analysis. Laboratories often retain serum samples for several days in case a retest is needed so immediate consultation with the clinical laboratory technician is essential to save the specimen for testing. Another sample collection technique involves collecting a blood or urine specimen at presentation to the ED but not performing the assay. This approach, sometimes called *toxicology hold*, allows collection of a specimen at a time when concentrations may be highest even if the need for the assay may not be clear.[13] The blood specimen can be refrigerated or the serum or urine can be frozen and assayed on request.

SPECIAL SITUATIONS

Other Biological Specimens

There is great interest in utilizing other biological specimen—such as hair, saliva, perspiration, and expired breath—and the application of POCTs for quantitative or qualitative analysis.[46] These are typically less invasive than venipuncture and some provide unique markers of long-term exposure or use (**Table 5-11**). Once a technology has been fully validated and sampling techniques refined to minimize interference, POCTs can be useful for drug screens at the bedside and worksite or longitudinal evaluation of chronic use (e.g., cocaine and marijuana in hair samples). In May 2015, SAMHSA proposed oral fluids (saliva) as an acceptable specimen for drug screening that meet federal regulatory requirements (see www.samhsa.gov/workplace/drug-testing for the final rule).[47] The FDA has approved several types of POCTs for clinical use. Although POCT results are typically available within 5–10 minutes, the test may be less accurate than laboratory-based analysis when the operator is not sufficiently trained and experienced with the particular POCT device.[24] A common and accepted application of POCTs is breath alcohol determination to assess driving impairment from ethanol use at the scene of an accident or arrest.[35]

Forensic and Legal Issues

In addition to clinical and regulatory applications for urine drug screens and serum drug concentrations, toxicological analysis has an important role in providing evidence for suspected cases of homicide, suicide, child abuse, drug-facilitated sexual assault ("drugged date rape"), environmental contamination, malpractice, workers' compensation, insurance claims, and product liability litigation. Chemical exposure monitoring of workers or the work environment requires specialized approaches such as long-term, onsite monitoring by an industrial hygiene specialist. Toxicological tests are also important in establishing brain death in patients being considered for organ donation or to remove life support. It is essential to establish that the apparent vegetative or unresponsive state is not due to drugs. Whenever legal action is anticipated, it is necessary to maintain a specimen chain-of-custody that can be documented as part of the evidence presentation.[10,13]

Postmortem analysis of biological specimens, such as gastric contents, organs, vitreous humor, bile, blood, and urine, can assist in the cause of death. These specimens are often collected at the time of autopsy, which may be days to weeks after death. The study of the changes that occur in drug distribution and metabolism after death has been called *postmortem toxicology*

TABLE 5-11. Characteristics of Selected Specimens for Toxicological Analysis[46]

SPECIMEN	STRENGTHS	WEAKNESSES	DETECTION TIMES
Urine	Available in sufficient quantities Higher concentrations of drugs or metabolites in urine than in blood Well-researched testing techniques Availability of POCTs	Specimen can be adulterated, substituted, or diluted May require observed collection Some individuals experience "shy bladder" syndrome and cannot produce a specimen Biological hazard for specimen handling and shipping to laboratory	Limited window of detection after drug use Typically 1–5 days, some substances are detected for 1–4 weeks
Hair	Observed and noninvasive specimen collection Good specimen stability (does not deteriorate) Convenient shipping and storage (no need to refrigerate) Difficult to adulterate or substitute	Few labs perform testing Costly and time consuming to prepare specimen for testing Difficult to interpret results Cannot detect alcohol use Will not detect very recent drug use (i.e., 7–10 days prior to test) Difficult to detect low-level use (e.g., single-episode)	Longest window of detection; best suited for chronic drug use Depends on hair length in the sample 1½ inch specimen reflects a 3-month history; hair grows about ½ inch per month
Oral fluids (saliva)	Observed and noninvasive specimen collection Minimal risk of tampering Samples can be collected easily in virtually any setting Availability of POCTs	Drugs and metabolites do not remain in oral fluids as long as they do in urine Limited specimen volume Requires supervision for 10–30 min before sampling Oral fluids contaminated by cannabinoids do not reflect presence in saliva and blood	Reflects recent drug use Approximately 10–24 hr
Sweat	Observed and noninvasive specimen collection Simple application and removal of skin patch Variable application time (generally 1–14 days) Difficult to adulterate	Few labs perform testing Risk of accidental or deliberate removal of patch External contamination of the patch may affect results Requires two visits (application and removal of patch)	Patch retains evidence of drug use for at least 7 days Detects low levels of some drugs two to five hr after last use

POCTs = point-of-care tests.

or *necrokinetics*. In addition to diffusion of some drugs to or from tissues and blood after death, the effects of putrefaction, fluid shifts on drug concentrations, and chemical stability need to be considered. This is an evolving field of study, which has already demonstrated that postmortem drug concentrations from various biological specimens may not always be appropriately referenced to drug concentration results derived from living humans.[48,49]

Pediatrics

The toxicological testing of newborn babies, preschool-age poisoning victims, and adolescents involves several unique ethical, technical, and societal concerns. Intrauterine drug exposure can lead to medical complications of newborns and such abuse may be confirmed by drug screens. When mothers do not admit to prepartal drug use, the routine screening of a newborn's urine or meconium poses economic and practical challenges, such as the cost of a generalized screening policy, difficulty in obtaining an adequate urine sample from a neonate and the preanalytical processing of meconium to make it suitable for analysis. Toxicological analysis of the mother's urine and the neonate's urine and meconium is helpful to identify substances that would lead to neonatal abstinence syndrome and to inform clinicians of its anticipated onset and duration.[50,51]

The use of urine drug screens in the pediatric ED provides minimally useful information because the offending agent is typically known and attempts at concealment are infrequent.[52] However, a broad or focused drug screen may be helpful for cases of suspected child abuse by poisoning or when the history of the poisoning is unclear.[50]

Prerequisite drug testing of adolescents for participation in school activities and routine screening by school officials, concerned parents, and pediatricians, presents several ethical dilemmas. Parents and school officials want assurance that substance abuse is not occurring, but the confidentiality and consent of the adolescent should be recognized. The American Academy of Pediatrics states that it "has strong reservations about testing adolescents at school or at home and believes that more research is needed on both safety and efficacy before school-based testing programs are implemented."[53] This policy statement is at odds with the desire of some parent groups to perform such tests without the adolescent's consent and with court rulings upholding a school's ability to perform mass or random drug tests of its students.[54] Drug use in school-age children has been associated with a variety of risk taking behaviors, such as carrying a gun, engaging in unprotected sexual intercourse with multiple partners, and suffering injury from a physical fight.[55] These behaviors may indicate the need for monitoring drug abuse with appropriate behavioral and health counseling. Many drug testing products are promoted for home use on the Internet, but their reliability and benefits are questionable.[56]

Sports and Drugs

Drugs are used by some amateur and professional athletes in hopes of enhancing athletic performance and by nonathletes to improve physical appearance. In 2013, an estimated 3.3% (range by state = 1.5 to 8.8%) of high school students (4.0% boys and 2.9% girls) have used androgenic anabolic steroids; of these students, nearly one third do not participate in sports but use steroids to change their appearance.[5,57] The types and variety of substances are typically different from those encountered in poisonings or substance abuse. Most workplace or clinical drug screens will not detect athletic performance enhancing drugs such as anabolic androgenic steroids, growth hormone, and erythroid-stimulating agents (**Table 5-12**).[58] The international term for drug use in sports is *doping,* and efforts to combat this practice are referred to as *doping control* or *antidoping.*

Governing athletic organizations, such as the International Olympic Committee, the United States Olympic Committee, and the National Collegiate Athletic Association, have established policies and analytical procedures for testing athletes as well as lists of banned substances. Most organized sports observe the World Antidoping Agency's guidelines for analytical tests, banned substances, and screening procedures.[59] The technical and scientific challenges in detecting many of these substances are unique to this field. Using banned drugs can result in an unfair, artificial advantage for competitive athletes and physical injury or permanent disability from the drug's effects, such as those from anabolic steroids.[57,58]

Prescription Drug Abuse

During the past 15 years the abuse of prescription drugs has risen dramatically and has increased in all age groups.[7] Nationwide 54.4 million (20.5%) of Americans aged 12 years and older reported taking prescription drugs for nonmedical purposes during their lifetime in 2014.[1] For the first time the number of poisoning deaths exceeded the number of motor vehicle traffic deaths in 2008. In 2014, drugs were the cause of 91% of these poisoning deaths with some type of opioid involved in 61% of the cases.[7] These trends have been recognized as a national epidemic.[60,61] In evaluating patients who may be abusing prescription drugs, a careful history, physical examination, and use of behavioral screening tools are important elements, which can be supported by drug screens.[46,62-64] A urine drug screen can assist in the detection of inappropriate use when there is sufficient suspicion. If a patient is on a methadone treatment program or is regularly receiving opioids for the relief of chronic pain, the urine drug screen should produce positive results. A negative finding could suggest poor adherence and possible diversion (**Minicase 7**). Characteristics of the drug (e.g., short duration of action) and the assay (e.g., ability to detect some synthetic opioids) should be considered before discussing the issue with the patient. In a national sample of pediatricians and family physicians, a survey indicated that 48% followed no defined procedures to ensure proper collection of the urine sample and most had poor responses on knowledge of drugs that are detected and those that could cause false-positive results. The authors concluded that primary care physicians are not prepared to assist with drug testing programs.[65] Knowledge and training at primary care settings on urine drug screening would enhance the ability to detect and help patients who are abusing prescription drugs and help stem the rise in drug abuse, diversion, and deaths.[46,66] Differences in federal workplace testing and the clinical setting, such as specimen type, collection procedures, drug testing panel, cutoff concentrations, and MRO involvement, should be recognized.[10,46,67]

TABLE 5-12. Some Purported Athletic Performance-Enhancing Substances

Amino acids (e.g., arginine, ornithine, lysine, aspartate, glutamine, leucine, tryptophan, carnitine)
Amphetamines[a]
Androgenic anabolic steroids
Androstenedione
Antiestrogenic agents (e.g., anastrozole, tamoxifen, clomiphene, fulvestrant)
Antioxidants (e.g., megadoses of ascorbic acid [vitamin C], tocopherol [vitamin E], β-carotene)
β-adrenergic blockers
β-2 agonists
Caffeine
Clenbuterol
Cocaine[a]
Corticotropin (ACTH)
Creatine
Designer anabolic steroids
Dehydroepiandrostenedione (DHEA)
Diuretics
Erythropoietin
Ethanol
γ-hydroxybutyrate (GHB)
Human chorionic gonadotropin
Human growth hormone
Selective androgen receptor modulators
Theophylline

[a] Can be detected on most routine immunoassay urine drug screens; the others are not typically detected on routine drug screens.

MINICASE 7

Monitoring Chronic Therapy with Opioid Analgesics

Alex P., a 32-year-old day laborer, developed chronic pain after a lower back injury. He currently has a prescription for a long-acting morphine preparation. Alex P. has had a history of anxiety and substance abuse of a variety of illicit and prescription drugs. He is otherwise healthy. During the past 24 months on this medication, he has lost his prescription twice and asked for a replacement. His physician has also written additional prescriptions at times when he complained that the dosage was inadequate to provide pain relief. His current daily total dose of morphine is 120 mg. He was recently arrested for forging a prescription to obtain additional opioid medication. Alex P. has had several random urine drug screens during the past 18 months that have been positive for morphine. On one occasion, the test result was positive for oxycodone for which he had a prescription from another physician.

QUESTION: How can urine drug screens be helpful in assessing adherence to an opioid analgesic regimen and identifying potential abuse of opioids?

DISCUSSION: The opioid drug treatment of noncancer-related chronic pain can be complicated by many factors, such as the need for escalating doses for adequate pain relief; potential for diversion of the drugs by selling or giving the drugs to others; abuse of the drugs to get high or satisfy a drug craving; and the risks of overdose and death. One of the approaches to monitor chronic therapy with opioids is to perform random drug screens. A positive drug screen can support that the person is taking the drug, while a negative result should raise the question of whether the person is diverting it to others or has stopped taking it for some reason. A drug screen that shows illicit, nonprescribed drugs or prescriptions from multiple or other prescribers, such as oxycodone in this case, strongly suggests that abuse of other medications is taking place, which all raise the risks for overdose and death. The physician is faced with several significant signals in this case that need attention to provide safe and effective care. If the physician is not a pain specialist, referral to one for this patient's chronic pain care should be considered.

SUMMARY

Testing for substance abuse, poisonings, and overdose affects society at several levels. Knowledge of assay limitations, sampling procedures, interfering substances, patient factors, and regulatory requirements will aid in the interpretation of the value of the test and its clinical relevance. In this chapter, several approaches and applications are discussed, but other situations that involve potential toxins—such as environmental contamination, chemical terrorism, and product safety testing—call for different approaches and pose unique challenges.

MINICASE 8

Where's the LSD?

In a backyard with two friends, Jacob C., an 18-year-old male, began exhibiting bizarre, agitated, hyperactive behavior 30 minutes after putting two paper blotters on his tongue, which were believed to contain LSD (lysergic acid diethylamide). When he soon exhibited seizure activity lasting 15–20 minutes, an ambulance was called to the scene. Tonic-clonic seizures continued during transport and hospitalization for another hour despite aggressive therapy to stop the seizures. During the next 36 hours, Jacob C. exhibited tachycardia, hyperthermia, acidosis, hypertension, renal failure, and minimal responsiveness. A CT (computerized tomography) scan of his head indicated cerebral edema. A urine drug screen on admission was positive for marijuana and benzodiazepines, the latter from drugs used to manage his seizures. After a complicated hospital course, his pupils became pinpoint and minimally responsive. An MRI (magnetic resonance imaging) indicated anoxic brain injury. He expired on the fifth day of hospitalization. At autopsy, anoxic brain injury was confirmed, and a sophisticated analysis of his blood, urine, and tissues revealed the presence of a designer hallucinogenic amphetamine, 2C-1NBOMe.

QUESTION: Why wasn't the designer amphetamine detected in the initial urine drug screen?

DISCUSSION: Only drugs or substances that are part of the drug screen analysis can be detected when considering all of the potential limitations of a false-negative or false-positive result. In this case, an immunoassay was used for a stat urine drug screen that only included the substances required by SAMHSA (Table 5-5). Marijuana and benzodiazepines are included in this panel, but LSD is not. In this case knowing the identity of the substance would not have changed the symptomatic and resuscitative treatment he appropriately received. When the medical examiner pursued a more extensive analysis at autopsy, the designer drug was identified. No LSD was detected. This patient's friends had purchased the 2C-1NBOMe as a "research chemical" on the Internet thinking it was a form of LSD.

Sources of Information

Because test characteristics vary with the type of test, manufacturer, assay kit, setting, and application, information about a specific test is critical for proper utilization and interpretation. Good communication with laboratory technicians is an important first step in ensuring proper testing. Laboratory technicians can provide guidance on sample collection, cutoff values, interfering substances, and other technical aspects. The package insert for immunoassays or other commercial assay kits is an important and specific guide to assay performance and known interfering substances with the specific assay. There are several textbooks that can be helpful in understanding techniques, values, and interfering substances.[68-70] Clinical toxicologists in poison control centers (list at www.aapcc.org or contact the local program at 1-800-222-1222 nationwide) can also provide useful information on laboratory tests particularly as they relate to poisonings. Several relevant publications are available at Internet websites of governmental agencies, such as the SAMHSA (www.samhsa.gov), the Office of Drug Policy Control (www.whitehouse.gov/ondcp), and the U.S. Drug Enforcement Administration (www.dea.gov). These websites can also increase awareness of persistent or emerging drugs of abuse **(Minicase 8)**. Quickviews of eight common urine drug screens by immunoassay include information on the signs and symptoms of these agents following abuse and overdose.[9,11,24,26,71,72]

LEARNING POINTS

1. ***How long does it take for a drug to clear the body and result in a negative urine drug screen?***

   ANSWER: It depends on a number of factors. Length of time for detection will vary with the sensitivity of the assay, whether the assay is directed to the parent drug or the metabolite, whether the drug or its metabolites exhibit extensive distribution to tissues, the dose of the drug taken, and whether the drug was used chronically or only once.

2. ***What does a negative result from a drug screen mean?***

   ANSWER: A negative result does not mean that the drug was not present or not taken; it means that it was not detected. Some reasons include that the drug may not have been part of the testing panel; the urine may have been too dilute for detection; the urine may have been collected before the drug was excreted in the urine; the urine sample may have been adulterated after collection to mask or avoid detection; or the specimen was obtained too late after the exposure.

3. ***How can serum concentrations be useful in the treatment of poisoned patients?***

   ANSWER: When a poisoning or overdose is suspected, a serum concentration is obtained when it can confirm the diagnosis of a poisoning when in doubt, aid the interpretation of a qualitative urine drug screen, determine whether antidotal therapy is indicated, or determine the effectiveness of a therapy. For assays not primarily intended for overdoses or poisonings (e.g., acetaminophen), drug concentrations occasionally are measured when the assay is used for another application such as therapeutic drug monitoring. In clinical settings, serum is typically not used for drug screening.

4. ***What should you do if the results do not make sense?***

   ANSWER: Consider actions that include checking the report and units of measure, talking with the laboratory technician, checking the package insert of the assay, searching the literature, seeking alternative causes of symptoms, and repeating the assay at the same or different laboratory.

5. ***Why are there so few drugs and substances on drug screens?***

   ANSWER: Whether a drug or substance is on a drug screen depends on several factors such as the following. The entity paying or requiring the drug screen can include as many drugs on the panel as financially and technically feasible. The SAMHSA panel (Table 5-5) reflects several common drugs of abuse and has been updated periodically through the federal regulatory process. Employers can and do add other drugs of concern for pre-employment, random monitoring, and accident investigation situations. Clinicians can often request or choose from a menu of drug panels to meet the needs of the patient situation or purpose, such as monitoring specific opioid analgesic use, misuse, and adherence. Assays beyond the routine menu are typically performed at a reference laboratory and require a longer turnaround time. In forensic settings, more extensive and sophisticated analytical techniques are often used to identify substances that are compared to an analytical library of known substances. A novel substance, such as a designer drug, poses significant analytical challenges to develop a procedure specific for the substance to identify its chemical composition. The decision to pursue analysis of a specific drug or substance is based on the consideration of the need for analysis, assay availability, timeliness, and cost. A key question to ask is "How will the results be useful for the particular circumstance?"

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QUICKVIEW | Urine Drug Screen, Amphetamines, and Methamphetamine

PARAMETER	DESCRIPTION	COMMENTS
Critical Value	Positive	Check for possible interferents; confirm result with confirmatory test such as GC-MS
Major causes of…		
Positive results	Following ingestion, intranasal application, injection, smoking (methamphetamine)	
Associated signs and symptoms	None may be evident at time of specimen collection; may involve exposure to illicit substances; may be used for legitimate purposes or abuse may involve exposure to illicit forms	Typical symptoms include CNS stimulation, euphoria, irritability, insomnia, tremors, seizures, paranoia, and aggressiveness; overdoses cause hypertension, tachycardia, stroke, arrhythmias, cardiovascular collapse, rhabdomyolysis, and hyperthermia
After use, time to…		
Negative result from light, sporadic use	2–5 days; clearance is faster in acidic urine	Methylphenidate typically will not be detected
Negative result from chronic use	Up to 2 weeks	
Possible spurious positive results with immunoassays	Ephedrine, pseudoephedrine, ephedra (ma huang), phenylephrine, selegiline, chlorpromazine, promethazine, trazodone, bupropion, desipramine, trimipramine, ritodrine, amantadine, ranitidine, phenylpropanolamine, brompheniramine, isometheptene, labetalol, phentermine, methylphenidate, isoxsuprine, trimethobenzamide, 3,4-methylenedioxy-N-methamphetamine (MDMA, Ecstacy)	A false-positive result may be caused by patient's use of drugs and dietary supplements; verify possible false positive with laboratory and assay package insert

CNS = central nervous system; GC-MS = gas chromatography/mass spectrometry; MDMA = 3,4-methylenedioxy-N-methamphetamine.

QUICKVIEW | Urine Drug Screen, Barbiturates

PARAMETER	DESCRIPTION	COMMENTS
Critical Value	Positive	Check for possible interferents; confirm result with confirmatory test such as GC-MS
Major causes of…		
Positive results	Following ingestion; rarely injected or used as a suppository	
Associated signs and symptoms	None may be evident at time of specimen collection; may involve exposure to medicines used for legitimate purposes or abuse	Typical symptoms include sedation; overdoses cause coma, ataxia, nystagmus, depressed reflexes, hypotension, and respiratory depression; consider coingestion of ethanol; primidone is metabolized to phenobarbital
After use, time to…		
Negative result from light, sporadic use	1–7 days	Depends on drug and extent and duration of use
Negative result from chronic use	1–3 weeks	Phenobarbital may be detected up to 4 weeks after stopping use
Possible spurious positive results with immunoassays	Ibuprofen, naproxen	Verify possible false positive with laboratory and assay package insert

GC-MS = gas chromatography/mass spectrometry.

QUICKVIEW | Urine Drug Screen, Benzodiazepines

PARAMETER	DESCRIPTION	COMMENTS
Critical Value	Positive	Check for possible interferents; confirm result with confirmatory test such as GC-MS
Major causes of…		
Positive results	Following ingestion or injection	Benzodiazepines vary in cross-reactivity and detectability
Associated signs and symptoms	None may be evident at time of specimen collection; may involve exposure to medicines used for legitimate purposes or abuse; may involve exposure to illicit forms	Typical symptoms include drowsiness, ataxia, slurred speech, sedation; oral overdoses can cause tachycardia and coma with rare severe respiratory or cardiovascular depression; rapid IV use can cause severe respiratory depression; consider coingestion of ethanol
After use, time to…		
Negative result	Typically up to 2 weeks; up to 6 weeks with chronic use of some agents	Some benzodiazepines may persist for a longer period of time and some have an active metabolite that may or may not be detected; flunitrazepam may not be detected; not all benzodiazepines will be detected by all immunoassays
Possible spurious positive results with immunoassays	Oxaprozin, sertraline	Verify possible false positive with laboratory and assay package insert

GC-MS = gas chromatography/mass spectrometry; IV = intravenous.

QUICKVIEW | Urine Drug Screen, Benzoylecgonine (cocaine metabolite)

PARAMETER	DESCRIPTION	COMMENTS
Critical Value	Positive	Check for possible interferents; confirm result with confirmatory test such as GC-MS
Major causes of…		
Positive results	Following snorting, smoking, injection, topical application (vagina, penis) or rectal insertion; possible passive inhalation; ingestion	
Associated signs and symptoms	None may be evident at time of specimen collection with heavy or chronic use; may involve exposure to medicines used for legitimate purposes or abuse; may involve exposure to illicit forms	Typical symptoms include CNS stimulation that produces euphoric effects and hyperstimulation such as dilated pupils, increased temperature, tachycardia and hypertension; overdoses cause stroke, acute myocardial infarction, seizures, coma, respiratory depression, arrhythmias
After use, time to…		
Negative result from light, sporadic use	12–72 hr	Cross-reactivity with cocaethylene (metabolic product of concurrent cocaine and ethanol abuse) varies with the assay
Negative result from chronic use	Up to 1–3 weeks	
Possible spurious positive results with immunoassays	Topical anesthetics containing cocaine; coca leaf tea	False finding for abuse False positives from caine anesthetics (e.g., lidocaine, procaine, benzocaine) are unlikely; verify possible false positive with laboratory and assay package insert

CNS = central nervous system; GC-MS = gas chromatography/mass spectrometry.

QUICKVIEW | Urine Drug Screen, Delta-9-tetrahydrocannabinol-9-carboxylic Acid

PARAMETER	DESCRIPTION	COMMENTS
Critical Value	Positive	Check for possible interferents; confirm result with confirmatory test such as GC-MS
Major causes of…		
Positive results	Following smoking, ingestion, possible passive inhalation	May be caused by sodium phosphate used as drug screen adulterant; patients taking dronabinol will also have positive test results
Associated signs and symptoms	None may be evident at time of specimen collection with heavy or chronic use; may involve exposure to illicit substances; may involve exposure to medicine used for legitimate purposes or abuse	Typical symptoms include delirium, conjunctivitis, food craving; other effects include problems with memory and learning, distorted perception, difficulty in thinking and problem solving, loss of coordination, sedation, and tachycardia
After use, time to…		
Negative result from light, sporadic use	7–10 days	
Negative result from chronic use	6–8 weeks typically, up to 3 months possible	May persist for a longer period of time with heavy, long-term use
Possible spurious positive results with immunoassays	Ibuprofen, naproxen, tolmetin, efavirenz, pantoprazole	False-positive result
		False positive for abuse; verify possible false positive with laboratory and assay package insert

GC-MS = gas chromatography/mass spectrometry.

QUICKVIEW | Urine Drug Screen, LSD

PARAMETER	DESCRIPTION	COMMENTS
Critical Value	Positive	Check for possible interferents; confirm result with confirmatory test such as GC-MS
Major causes of…		
Positive results	Following ingestion, placement in buccal cavity or ocular instillation	Not well-absorbed topically
Associated signs and symptoms	May involve exposure to illicit substances	Typical symptoms include unpredictable hallucinogenic effects; physical effects include mydriasis, elevated temperature, tachycardia, hypertension, sweating, loss of appetite, sleeplessness, dry mouth, and tremors; flash-backs months later are possible
After use, time to…		
Negative result	24–48 hr typically, up to 120 hr possible	
Possible spurious positive results with immunoassays		LSD is a schedule I drug with no legitimate routine medical use; verify possible false positive with laboratory and assay package insert

GC-MS = gas chromatography/mass spectrometry; LSD = lysergic acid diethylamide.

QUICKVIEW | Urine Drug Screen, Opiates

PARAMETER	DESCRIPTION	COMMENTS
Critical Value	Positive	Check for possible interferents; confirm result with confirmatory test such as GC-MS
Major causes of…		
Positive results	Following ingestion, injection, dermal application of drug-containing patches, rectal insertion	Synthetic opioids (e.g., fentanyl, fentanyl derivatives, meperidine, methadone, pentazocine, and tramadol) have minimal cross-reactivity and may not be detected
Associated signs and symptoms	None may be evident at time of specimen collection; may involve exposure to illicit substances; may involve exposure to medicines used for legitimate purposes or abuse; ingestion of large amounts of food products made with poppy seeds	Typical symptoms include CNS depression, drowsiness, miosis, constipation; overdoses cause coma, hypotension, respiratory depression, pulmonary edema, and seizures
		Heroin use is confirmed by the presence of 6-acetylmorphine (6-AM)
After use, time to…		
Negative result	2–3 days typically, up to 6 days with sustained-release formulations, up to 1 week with prolonged or heavy use	
Possible spurious positive results with immunoassays	Poppy seeds	False positive for drug abuse
	Rifampin, some fluoroquinolones, quinine	False-positive result; consider patient's legitimate use of opioid analgesics including long-term pain management and opioid withdrawal treatment with methadone, or buprenorphine; verify possible false positive with laboratory and assay package insert

CNS = central nervous system; GC-MS = gas chromatography/mass spectrometry.

QUICKVIEW | Urine Drug Screen, PCP

PARAMETER	DESCRIPTION	COMMENTS
Critical Value	Positive	Check for possible interferents; confirm result with confirmatory test such as GC-MS
Major causes of…		
Positive results	Following ingestion, smoking, snorting, or injection	
Associated signs and symptoms	None may be evident at time of specimen collection; may involve exposure to illicit substances; may involve exposure to medicines containing dextromethorphan or diphenhydramine used for legitimate purposes or abuse	Typical symptoms include hallucinations, schizophrenia-like behavior, hypertension, elevated temperature, diaphoresis, tachycardia; high doses cause nystagmus, ataxia, hypotension, bradycardia, depressed respirations, seizures, and coma
After use, time to…		
Negative result from light, sporadic use	2–10 days	May persist for a longer period of time with heavy, long-term use or massive overdose preceded by chronic use
Negative result from chronic use	Weeks or months	
Possible spurious positive results with immunoassays	Ketamine, dextromethorphan, diphenhydramine, imipramine, mesoridazine, thioridazine, venlafaxine, ibuprofen, meperidine, tramadol	False-positive result; verify possible false positive with laboratory and assay package insert

GC-MS = gas chromatography/mass spectrometry; PCP = phencyclidine.

6

INTERPRETATION OF SERUM DRUG CONCENTRATIONS

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OBJECTIVES

After completing this chapter, the reader should be able to

- Justify the need for concentration monitoring of a drug based on its characteristics and the clinical situation
- Identify and justify information needed when requesting and reporting drug concentrations
- Describe and categorize factors that may contribute to interpatient variation in a therapeutic range of drug concentrations
- Explain the importance of documenting the time a sample is obtained relative to the last dose, as well as factors that can affect interpretation of a drug concentration, depending on when it was obtained
- Compare linear to nonlinear pharmacokinetic behavior with respect to how drug concentration measurements are used to make dosage regimen adjustments
- Describe how altered serum binding, active metabolites, or stereoselective pharmacokinetics can impact the interpretation of drug concentration measurements

The pharmacist is a key member in the therapeutic drug monitoring process. This chapter is designed to review the indications for drug concentration monitoring and to discuss how drug concentrations obtained from the clinical laboratory, specialized reference laboratory, or physician's office should be interpreted. General considerations for interpretation will be described, as well as unique considerations for drugs that commonly undergo therapeutic drug monitoring. Future directions of therapeutic drug monitoring will also be discussed.

This chapter is not intended to provide an in-depth review of pharmacokinetic dosing methods; nevertheless, knowledge of certain basic pharmacokinetic terms and concepts is expected. The general phrase *drug concentration* will be used throughout the chapter unless specific references to serum, plasma, whole blood, and saliva are more appropriate. The bibliography lists numerous texts about therapeutic drug monitoring and clinical pharmacokinetic principles with applications to clinical practice.

THERAPEUTIC DRUG MONITORING

Therapeutic drug monitoring is broadly defined as the use of drug concentrations to optimize drug therapy for individual patients.[1] Prior to using drug concentrations to guide therapy, physicians adjusted drug doses based on their interpretation of clinical response. In many cases, drug doses were increased until obvious signs of toxicity were observed (e.g., nystagmus for phenytoin or tinnitus for salicylates). The idea that intensity and duration of pharmacologic response depended on serum drug concentration was first reported by Marshall and then tested for the screening of antimalarials during World War II.[2,3] Koch-Weser, in a hallmark paper, described how steady-state serum concentrations of commonly used drugs can vary 10-fold among patients receiving the same dosage regimen.[4] He further described how serum concentrations predict intensity of therapeutic or toxic effects more accurately than dosage.

Starting in the 1960s, there was rapid improvement in analytical methods used for drug concentration measurements; extensive research correlating serum or plasma drug concentrations with clinical efficacy and toxicity quickly followed. In the 1970s, physicians, pharmacists, and laboratory technologists began forming specialized therapeutic drug monitoring or clinical pharmacokinetics services in hospitals. Today, with the emergence of immunoassays that require no specialized equipment, drug concentration measurements can be easily performed in physician offices.[5]

The increased availability and convenience of drug assay methods has led to a number of concerns. Is therapeutic drug monitoring being done simply because it is available, rather than because it is clinically necessary? There are numerous reports of suboptimal therapeutic drug monitoring practices that contribute to inappropriate decision making as well as wasted resources.[6-8] Questions are also being raised about whether therapeutic drug monitoring actually improves patient outcomes.[9,10] However, many clinicians claim that therapeutic drug monitoring is greatly underused and could, if appropriately used, further improve patient care and reduce healthcare costs.[11-13] Clearly, there is a need for more education of all healthcare professionals involved in the therapeutic drug monitoring process to make its use more appropriate and cost-effective. Such education efforts have been shown to effectively reduce the numbers of inappropriate drug concentration requests.[14]

Goal and Indications for Drug Concentration Monitoring

The primary goal of therapeutic drug monitoring is to maximize the benefit of a drug to a patient in the shortest possible time with minimal risk of toxicity. The number of hospitalizations or office visits used to adjust therapies or manage and diagnose adverse drug reactions may therefore be reduced, resulting in cost savings.

Drug concentration measurements should not be performed unless the result will affect some future action or decision. Monitoring should not be done simply because the opportunity presents itself; it should be used discriminatingly to answer clinically relevant questions and resolve or anticipate problems in drug therapy management.[15] The clinician should always ask, "Will this drug concentration value provide more information to me than sound clinical judgment alone?"[9] The following are examples of clinical situations and the clinical questions that drug concentration measurements might be able to answer:

- **Therapeutic confirmation**—A patient is on a regimen that appears to offer maximum benefit with acceptable side effects. *Question: What is the drug concentration associated with a therapeutic effect in this patient for future reference?*
- **Dosage optimization**—A patient has a condition in which clinical response is not easily measured and has been initiated on a standard regimen of a drug. There is modest improvement and no symptoms of toxicity are evident. *Question: Can I increase the dose rate to further enhance effect? If so, by how much?*
- **Confirmation of suspected toxicity**—A patient is experiencing certain signs and symptoms that could be related to the drug. *Question: Are these signs and symptoms most likely related to a dose rate that is too high? Can I reduce the daily dose, and, if so, by how much?*
- **Avoidance of inefficacy or toxicity**—A patient is initiated on a standard regimen of an antibiotic that is known to be poorly absorbed in a small percentage of patients. Sustained subtherapeutic concentrations of this drug can lead to drug resistance. *Question: Will a higher daily dose be needed in this patient?* A patient has been satisfactorily treated on a dosage regimen of Drug A. The patient experiences a change in health or physiologic status or a second drug, suspected to interact with Drug A, is added. *Question: Will a dosage regimen adjustment be needed to avoid inefficacy or toxicity?*
- **Distinguishing nonadherence from treatment failure**—A patient has not responded to usual doses and noncompliance is a possibility. *Question: Is this a treatment failure, or does the patient need counseling on adherence?*

Characteristics of Ideal Drugs for Therapeutic Drug Monitoring

Not all drugs are good candidates for therapeutic drug monitoring, no matter how appropriate the indication seems to be. Those for which drug concentration monitoring will be most useful have the following characteristics[16]:

- **Readily available assays**—Methods for drug concentration measurement must be thoroughly evaluated for sensitivity, specificity, accuracy, and precision and be available to the clinician at a cost to justify the information to be gained. Chromatographic methods are most likely used in laboratory settings and are considered in many cases to be the reference methods. Increased interest in methods for use in ambulatory settings, however, has led to the development of immunoassay systems purported to be fast, reliable, and cost-effective.[5,18-21]
- **Lack of easily observable, safe, or desirable clinical endpoint**—Clinically, there is no immediate, easily monitored, and predictable clinical parameter to guide dosage titration. For example, waiting for arrhythmias or seizures to occur or resume may be an unsafe and undesirable approach to dosing antiarrhythmics and antiepileptics.
- **Dangerous toxicity or lack of effectiveness**—Toxicity or lack of effectiveness of the drug presents a danger to the patient. For example, serum concentrations of the antifungal drug, flucytosine, are not routinely monitored. However, specialized monitoring may be done to ensure that concentrations are below 100 mg/L to avoid gastrointestinal side effects, blood dyscrasias, and hepatotoxicity. As another example, specialized monitoring of the protease inhibitors (PIs) may be done to ensure adequate concentrations because rapid emergence of antiviral resistance is observed with sustained exposure to subtherapeutic concentrations.
- **Unpredictable dose–response relationship**—There is an unpredictable dose–response relationship, such that a dose rate producing therapeutic benefit in one patient may cause toxicity in another patient. This would be true for drugs that have significant interpatient variation in pharmacokinetic parameters, drugs with nonlinear elimination behavior, and drugs with pharmacokinetic parameters that are affected by concomitant administration of other drugs. For example, patients given the same daily dose of phenytoin can demonstrate a wide range of serum concentrations and responses.
- **Narrow therapeutic range**—The drug concentrations associated with therapeutic effect overlap considerably with the concentrations associated with toxic effects, such that the zone for therapeutic benefit without toxicity is very narrow. For example, the therapeutic range of total serum concentrations of phenytoin is widely accepted to be 10–20 mg/L for most patients; the upper limit of the range is only twice the lower limit.

- **Good correlation between drug concentration and efficacy or toxicity**—This criterion must apply if we are using drug concentrations to adjust the dosage regimen of a drug. For example, a patient showing unsatisfactory seizure control with a serum phenytoin concentration of 8 mg/L is likely to show improved control with a serum concentration of 15 mg/L.

Other than availability of an assay, it may not be necessary for a drug to fulfill all of the above characteristics for drug concentration monitoring to help guide clinical decision making. Newer drugs that do not yet have clearly defined therapeutic ranges may be monitored only under special circumstances (e.g., to ensure adherence). Other drugs may not have a clearly defined upper or lower limit to the therapeutic range but are monitored under special circumstances to ensure efficacy or avoid toxicity. This goes back to the importance of the drug concentration for answering a specific clinical question: Will the information provided by this measurement help to improve the patient's drug therapy?

Information Needed for Planning and Evaluating Drug Concentrations

Drug concentrations should be interpreted in light of full information about the patient, including clinical status. Information about the timing of the sample relative to the last dose is especially critical and is one of the biggest factors making drug concentrations unusable or cost-ineffective.[6,22-24] **Table 6-1** provides a list of the essential information needed for a drug concentration request. Laboratory request forms or computer entry forms must be designed to encourage entry of the most important information. All relevant information should be included on both the request form and the report form to facilitate accurate interpretation. It is particularly important to verify the time of sample draw because phlebotomists or

TABLE 6-1. Information Needed on Laboratory Request Form

TYPE OF DATA	SPECIFIC DATA	WHY NECESSARY
Patient identification	• Name, address, identification number, and physician name	All blood samples look alike and could easily be switched among patients without appropriate identification
Patient demographics and characteristics	• Age, gender, ethnicity, height, weight, and pregnancy	The therapeutic range for a given drug may depend on the specific indication being treated (e.g., digoxin for atrial arrhythmias versus heart failure); if there is no history of prior drug concentration measurements, information about concurrent disease states, physiologic status, and social habits may help with initial determination of population pharmacokinetic parameters, in order to determine if the resulting concentration is expected or not; information about renal function and albumin is important if a total drug concentration is being measured for a drug normally highly bound to serum proteins; it is also important to know if any endogenous substances due to diseases will interfere with the assay; electrolyte abnormalities may affect interpretation of a given concentration (e.g., digoxin)
History and physical examination	• Condition being treated • Organ involvement (renal, hepatic, cardiac, gastrointestinal, and endocrine) • Fluid balance and nutritional status • Labs (albumin, total protein, liver function enzymes, INR, bilirubin, serum creatinine or creatinine clearance, thyroid status, and electrolyte abnormalities) • Smoking and alcohol history	
Specimen information	• Time of collection • Nature of specimen: blood, urine, or other body fluid site of collection • Order of sample, if part of a series • Type of collection tube • Time of receipt by laboratory	Laboratories often retain samples for several days and detailed information will help to find a sample if important pre-, post-, or random samples are needed; the time of collection relative to the dose is extremely important for proper interpretation; (Close to a trough? Closer to a peak?) knowing the type of collection tube is important because of the many interferences that may occur; it is important to know the collection site relative to the administration site, if intravenous route is used; if a series of samples is to be drawn, the labeled timing of the collection tubes can get mixed up
Drug information	• Name of drug to be assayed • Current dosage regimen, including route • Type of formulation (sustained-release, delayed-release, or prompt-release) • Length of time on current regimen • Time of last dose • Concurrent drug therapy • Duration of intravenous infusion	It is important to know if the concentration was drawn at a steady state and when the concentration was drawn relative to the last dose; it is also important to know if there are any potential drug interferences with the assay to be used
Drug concentration history	• Dates and times of prior concentration measurements • Response and drug regimen schedules associated with prior concentrations	It is important to know what drug concentrations have been documented as effective or associated with toxicity; it is also important to know how drug concentrations have changed as a consequence of dosage regimen
Purpose of assay and urgency of request	• Therapeutic confirmation • Suspected toxicity • Anticipated inefficacy or toxicity due to change in physiologic/health status or drug–drug interaction • Identification of drug failure • Suspected overdose	This forces the clinician to have a specific clinical question in mind before ordering a sample; it also aids in the interpretation of results

INR = international normalized ratio.
Source: Adapted with permission from references 16 and 17.

TABLE 6-2. Common Reasons Why Drug Concentration Results Do Not Make Sense

CATEGORY OF FACTOR	SPECIFIC EXAMPLES
Related to drug administration or blood sampling logistics	• Wrong dose or infusion rate administered • Dose skipped or infusion held for a period of time • Dose given at time other than recorded; blood drawn as ordered • Dose given at right time; blood drawn at time other than recorded • Sample taken through an administration line, which was improperly flushed prior to sample withdrawal • Sample taken from the wrong patient • Improper or prolonged storage prior to delivery to laboratory • Wrong collection tube/device used • Patient was dialyzed between doses
Related to pharmacokinetics	• Sample is drawn prior to steady-state attainment • Orders for digoxin samples are not clearly specified to be drawn at least six hours postdistribution • Samples are ordered at the wrong times relative to last dose to reflect specific needs (e.g., peaks and troughs) • Concentrations of active metabolites are not ordered when appropriate • Concentrations for total drug are ordered for a drug with unusual serum protein binding without recognition that the usual therapeutic range of total drug will not apply • Samples following intravenous administration are drawn prior to completion of distribution phase (e.g., vancomycin, aminoglycosides)
Related to the laboratory	• The wrong drug is assayed • Critical active metabolites are not assayed • Interferences or artifacts caused by endogenous substances (bilirubin, lipids, and hemolysis) or concurrent drugs • Improper or prolonged storage prior to assay • Technical errors with the assay
Related to the patient	• Patient is not adherent with therapy • Taking interacting medications that may increase or decrease a drug's concentration • Patient-specific laboratory parameters important for a drug's pharmacokinetic profile are altered (e.g., albumin)

Source: Adapted with permission from references 17 and 25.

computer-generated labels commonly identify samples with the time of the *intended* draw instead of the *actual* draw time. Some hospital laboratories have minimized the number of inappropriate samples by refusing to run any samples that are not accompanied by critical information, such as the timing of the sample relative to the last dose.[6] The laboratory report form should also include the assay used; active metabolite concentration (if measured); and parameters reflecting the sensitivity, specificity, and precision of the method.

Accuracy and completeness of the information provided on a laboratory request form is particularly important in light of the many problems that can occur during the therapeutic drug monitoring process. A drug concentration that seems to be illogical, given the information provided on the form, may be explained by a variety of factors as shown in **Table 6-2** (**Minicase 1**).

Considerations for Appropriate Interpretation of Drug Concentrations

To appropriately interpret a drug concentration, it is important to have as many answers as possible to the following questions:

- **Therapeutic range**—What do the studies show to be the usual therapeutic range? How frequently will patients show response at a concentration below the lower limit of the usual range? How frequently will patients show toxicity at a concentration below the upper limit of the usual range? What are the usual signs and symptoms indicating toxicity?
- **Sample timing**—Was the sample drawn at a steady state? Was the sample drawn at a time during the dosing interval (if intermittent therapy) that reflects the intended indication for monitoring (a peak, a trough, a "random" concentration, or an average concentration)? During the dosing interval, when is a peak concentration most likely to occur for the formulation administered? Does the formulation exhibit a lag time for release or absorption, such that the lowest concentration will occur into the next dosing interval?
- **Use of concentrations for dosage adjustment**—Does the drug display first-order (linear) pharmacokinetic elimination behavior such that an increase in daily dose will produce a proportional increase in the average drug concentration? Will more complex adjustment methods be needed for drugs that display nonlinear elimination behavior? Is the dosage adjustment method focused on attaining specific peaks and troughs, or specific average concentrations?
- **Protein binding, active metabolites, and other considerations**—How are total drug concentrations in serum interpreted in cases of altered serum protein binding? How are concentrations or contributions of active metabolite considered along with parent drug? Is the drug administered as a racemic mixture and if so, do the enantiomers differ in activity and pharmacokinetic behavior? Do certain physiologic or pathologic conditions affect a patient's response to the drug at a given concentration?

Each of these categories will be described in general below and, more specifically, for each drug or drug class in the Applications section.

MINICASE 1

Importance of Documenting Drug Administration Times

Michael T., an 86-year-old male (95 kg, 178 cm, baseline SCr 0.78), is receiving vancomycin monotherapy for treatment of a gram-positive bacteremia (unknown source). According to the medical chart, he receives four doses of a vancomycin 2000 mg q-12-hr regimen infused over two hours on a schedule of 0700/1900. The estimated/predicted half-life of vancomycin in Michael T. based on estimated creatinine clearance is six hours. A concentration drawn at 0600 the following morning is reported as 16 mg/L. Based on the current information, the regimen of vancomycin 2000 mg q 12 hr is continued. A repeat concentration three days later at 0630 reveals a vancomycin trough concentration of 27 mg/L. Renal function, as indicated by creatinine clearance, has not changed in this patient. The pharmacist receives a call to assess and interpret this concentration. If accurate, a dosage adjustment will be necessary to avoid toxicities.

QUESTION: What are the possible explanations for apparent changes in serum vancomycin results? Which vancomycin concentration accurately reflects the current dosage regimen?

DISCUSSION: For any drug requiring therapeutic drug monitoring, one must first consider whether the concentrations accurately represent a steady state. With an estimated vancomycin half-life of six hours, a steady state should have been reached after four doses or 48 hours. Because vancomycin is highly dependent on the kidney for elimination, a second consideration would be renal function. Of note, this patient's creatinine clearance is unchanged. Thirdly, laboratory errors or assay interference/artifacts could lead to difficulty in interpretation of serum drug concentrations. In the case of aminoglycosides, for example, coadministration of piperacillin–tazobactam may lead to in vitro inactivation, which may lead to falsely subtherapeutic concentrations. However, no such interferences were noted for vancomycin in this case. Finally, it is important to confirm the accuracy of blood sampling or drug administration times. After investigating this patient's medication administration record further, it is discovered that his third vancomycin dose was held, and no adjustment to timing of orders was performed. For this reason, the measured concentration of 16 mg/L was in fact 24 hours after the last dose, therefore, not reflecting a true 12-hour trough on the 2000 mg q-12-hr regimen. After analysis of subsequent administration times and doses of vancomycin, the dose is adjusted to 2000 mg every 24 hours. If the first measured concentration had been initially noted to be drawn 24 hours after the previous dose, the clinician could have predicted an elevated vancomycin concentration on the q-12-hr regimen, and a dose adjustment would have been made at that time.

THE THERAPEUTIC RANGE

A *therapeutic range* (also referred to as a *therapeutic reference range*) is best defined as a "range of drug concentrations within which the probability of the desired clinical response is relatively high and the probability of unacceptable toxicity is relatively low."[1,26,27] This means that the reference range reported by a laboratory is actually a population-based average for which the majority of patients are expected to respond with acceptable side effects. Thus, there will always be some patients who exhibit therapeutic effect at drug concentrations below the lower limit, while others will experience unacceptable toxicity at concentrations below the upper limit. Therefore, a patient's therapy is always best guided by that patient's individual therapeutic concentration.[26,27]

Figure 6-1 illustrates how the probability of response and toxicity increases with drug concentration for a hypothetical drug and how a therapeutic range might be determined based

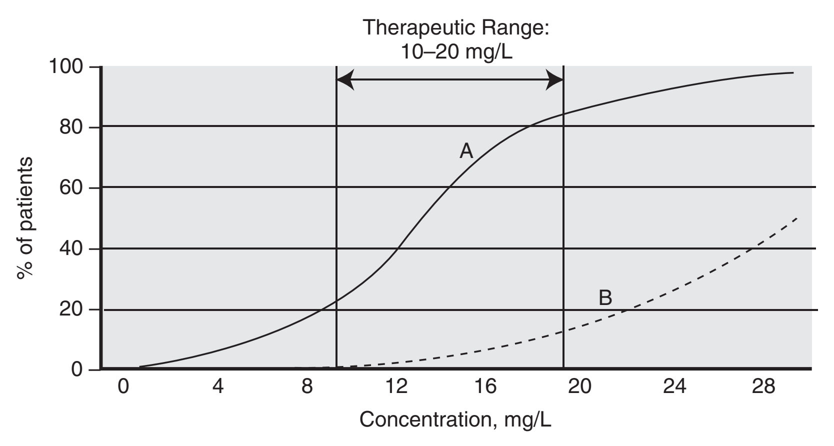

FIGURE 6-1. The therapeutic range for a hypothetical drug. Line A is the percentage of patients displaying a therapeutic effect; line B is the percentage of patients displaying toxicity.

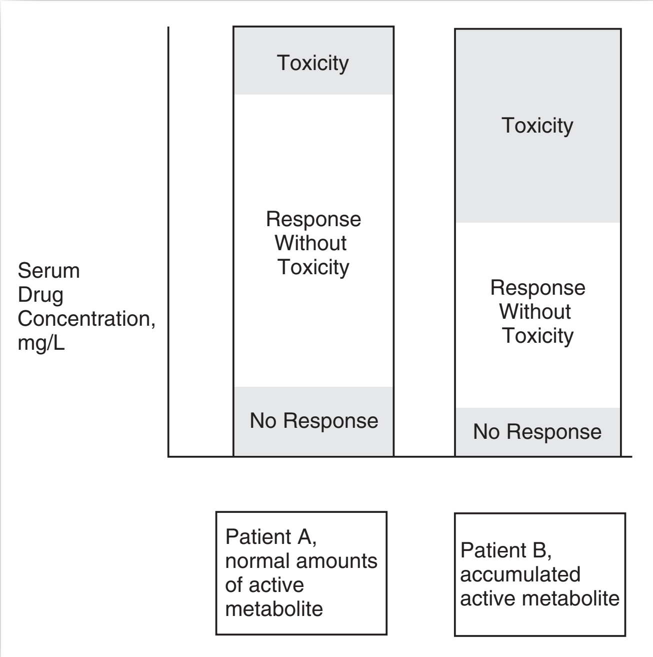

FIGURE 6-2. Representation showing how the individual therapeutic range of a hypothetical drug can differ in a patient with renal impairment because of accumulated active metabolite.

on these relative probabilities. **Figure 6-2** shows how patterns for response and toxicity can differ in two different patients receiving the same drug. If the hypothetical drug in question has an active metabolite that accumulates more than the parent drug in renal impairment, and if that metabolite contributes more to toxicity than to efficacy, then the individual therapeutic range in the patient with renal impairment will be narrower with lower concentrations. Concentration monitoring of the active metabolite would be especially important in that situation.

Drug concentration monitoring is often criticized by claims that therapeutic ranges are not sufficiently well defined.[11,12] The lack of clearly defined therapeutic ranges for older drugs is partially attributable to how these ranges were originally determined. Eadie describes the process that was typically used for determination of the therapeutic ranges of the antiepileptic drugs: "These ranges do not appear to have been determined by rigorous statistical procedures applied to large patient populations. Rather, workers seem to have set the lower limits for each drug at the concentration at which they perceived a reasonable (although usually unspecified) proportion of patients achieved seizure control, and the upper limit at the concentration above which overdosage-type adverse effects appear to trouble appreciable numbers of patients, the values then being rounded off to provide a pair of numbers, which are reasonably easy to remember."[15] In an ideal world, studies to define therapeutic ranges for drugs should use reliable methods for measurement of response and should be restricted to patients with the same diseases, age range, and concurrent medications.[1] In recent years, the U.S. Food and Drug Administration (FDA) has recognized the importance of determining concentration versus response relationships early during clinical trials.[28]

Anything that affects the pharmacodynamics of a drug, meaning the response at a given drug concentration, will affect the therapeutic range. These factors include the following:

- **Indication**—Drugs that are used for more than one indication are likely to be interacting with different receptors. Thus, a different concentration versus response profile might be expected depending on the disease being treated. For example, higher serum concentrations of digoxin are needed for treatment of atrial fibrillation as compared to congestive heart failure. Higher antibiotic drug concentrations may be needed for resistant organisms or to penetrate certain infected tissues.
- **Active metabolites**—As shown in Figure 6-2, variable presence of an active metabolite can shift the therapeutic range for that individual patient up or down. These metabolites may behave in a manner similar to the parent drug or may interact with different receptors altogether. In either case, the relationship between parent drug concentration and response will be altered.
- **Concurrent drug treatment**—In a manner similar to active metabolites, the presence of other drugs that have similar pharmacodynamic activities will contribute to efficacy or toxicity, but not to measurement of the drug concentration. The therapeutic range will be shifted.
- **Patient's age**—Although there is not much information concerning developmental changes in pharmacodynamics, it is believed that the numbers and affinities of pharmacologic receptors change with progression of age from newborns to advanced age.[29] This would be expected to result in a shift of the therapeutic range.
- **Electrolyte status**—As an example, hypokalemia, hypomagnesemia, and hypercalcemia are all known to increase the cardiac effects of digitalis glycosides and enhance the potential for digoxin toxicity at a given serum concentration.[30]
- **Concurrent disease**—As an example, patients with underlying heart disease (cor pulmonale, coronary artery disease) have increased sensitivity to digoxin.[30] There is also evidence that thyroid disease alters the usual response patterns of digoxin.[30]
- **Variable ratios of enantiomers**—Some drugs are administered as racemic mixtures of enantiomers, which may have different response/toxicity profiles as well as pharmacokinetic behaviors. Thus, a given concentration of the summed enantiomers (using an achiral assay method) will be associated with different concentrations of response or toxicity in patients with different proportions of the enantiomers. This has been extensively studied for disopyramide.[31]
- **Variable genotype**—There is growing evidence that response to certain drugs is genetically determined. For selected drugs, patients may be genotyped before starting drug treatment to identify them as nonresponders, responders, or toxic responders (Chapter 7).[32-34]
- **Variable serum protein binding**—Theoretically, only the unbound concentration of drug in blood is capable

of establishing equilibrium with pharmacologic receptors, thus making it a better predictor of response than total drug concentration. Most drug concentrations in serum, plasma, or blood, however, are measured as the summed concentration of bound and unbound drug. It is very likely that some of the patients who show toxicity within the conventional therapeutic range have abnormally low protein (e.g., albumin) binding and high concentrations of unbound drug in blood.[35] Low protein binding of a drug in blood can be the result of either reduced protein concentrations or the presence of other substances in blood that displace the drug from protein binding sites.

In summary, the therapeutic range reported by the laboratory is only an initial guide and is not a guarantee of desired clinical response in any individual patient. Every effort must be made to consider other signs of clinical response and toxicity in addition to the drug concentration measurement. Therapeutic ranges for the most commonly monitored drugs discussed in the Applications section of this chapter are reported in **Table 6-3.**

TABLE 6-3. Data to Aid Interpretation of Concentrations of Drugs That Are Commonly Monitored

	RECOMMENDED CONCENTRATIONS	RECOMMENDED TIMING	CONSIDERATIONS FOR INTERPRETATION: PROTEIN BINDING, ACTIVE METABOLITES, OTHER FACTORS
Bronchodilators			
Theophylline	Adult: 5–15 mg/L Children: 5–10 mg/L Neonate: 5–10 mg/L	Trough or $C_{ss,avg}$ Steady state occurs in 24 hr for an average adult nonsmoker receiving a maintenance infusion, but may take longer for sustained-release products	Concentrations up to 20 mg/L may be necessary in some patients; the caffeine metabolite is of minor significance in adults but may contribute to effect in neonates; theophylline has been replaced by safer bronchodilators in children, and by caffeine in neonates
Antiepileptics			
Carbamazepine	4–12 mg/L	Trough or $C_{ss,avg}$ Steady state may require up to 2–3 wk after initiation of full dose rate due to autoinduction	Lower total concentrations may be more appropriate in patients with decreased protein binding (liver disease, hypoalbuminemia, and hyperbilirubinemia), or in patients taking other anticonvulsants
Phenobarbital	10–40 mg/L	Anytime during interval; steady state may require up to 3 wk	Many drug interactions; consider impact on concentration when starting/stopping interacting medications
Phenytoin	Based on phenytoin concentrations: Adult: 10–20 mg/L (total) Infant: 6–11 mg/L (total) Neonate: 8–15 mg/L (total)	Trough or $C_{ss,avg}$ Steady state may require up to 3 wk	Measurement of unbound phenytoin concentrations (therapeutic range of 1–2 mg/L) may be preferred in most patients; lower total phenytoin concentrations may be more appropriate in patients with decreased protein binding due to hypoalbuminemia (e.g., liver disease, nephrotic syndrome, pregnancy, cystic fibrosis, burns, trauma, malnutrition, AIDS, and advanced age), end-stage renal disease, concurrent salicylic acid or valproic acid
Valproic acid	Epilepsy: 50–100 mg/L (total) Mania: 50–125 mg/L (total)	Trough or $C_{ss,avg}$ Steady state may require up to 5 days	Lower total valproic acid concentrations may be more appropriate in patients with hypoalbuminemia (liver disease, cystic fibrosis, burns, trauma, malnutrition, and advanced age), hyperbilirubinemia, end-stage renal disease, and concurrent salicylic acid; valproic acid shows interpatient variability in unbound fraction because of nonlinear protein binding; total concentrations will increase less than proportionately with increases in daily dose, while unbound concentrations will increase proportionately
Antimicrobial Drugs			
Amikacin	Traditional dosing: Peaks: 20–30 mg/L Troughs: <8 mg/L	Traditional dosing: steady state should be based on estimated half-life, particularly in patients with renal impairment Extended-interval dosing: per protocol Desired peak will depend on infection site (i.e., high inoculum infections necessitating higher peaks)	
Gentamicin, tobramycin	Traditional dosing: Peaks: 6–10 mg/L Troughs: <1–2 mg/L	Traditional dosing: steady state should be based on estimated half-life, particularly in patients with renal impairment Extended-interval dosing: per protocol	
Vancomycin	Troughs: 10–20 mg/L (intermittent dosing)	Trough, within 30 min–1 hr of next dose; steady state may require up to 2–3 days in patients with normal renal function	Benefit has been demonstrated with trough concentrations >10 mg/L in preventing vancomycin resistance; trough concentrations of 15–20 mg/L are recommended for high inoculum infections caused by *S. aureus* (e.g., bacteremia, endocarditis, osteomyelitis, meningitis, or pneumonia)

cont'd

TABLE 6-3. Data to Aid Interpretation of Concentrations of Drugs That Are Commonly Monitored, cont'd

	RECOMMENDED CONCENTRATIONS	RECOMMENDED TIMING	CONSIDERATIONS FOR INTERPRETATION: PROTEIN BINDING, ACTIVE METABOLITES, OTHER FACTORS
Antifungal Agents			
Posaconazole	Trough >1 mg/L	Concentration can be drawn at any time during dosing interval once steady state reached at end of first week of therapy	Variability in absorption and concentrations noted between different formulations (e.g., oral tablets versus oral suspension)
Voriconazole	Lower limit: >1 mg/L Upper limit: <4–6 mg/L	Trough concentration (e.g., prior to next dose) within first week of therapy initiation or dosage adjustments Steady state may be reached in 1–2 days; however, it is recommended to wait at least 5 days to measure trough concentration	
Cardiac Drugs			
Digoxin	0.5–2 mcg/L	NEVER sooner than 6 hr after an oral dose; steady state may require up to 7 days with normal renal function	Toxicity more likely within therapeutic range in patients with hypokalemia, hypomagnesemia, hypercalcemia, underlying heart disease, and hypothyroidism; patients with hyperthyroidism may be resistant at a given digoxin concentration, drug interactions
Cytotoxic Drugs			
Methotrexate	Therapeutic levels: variable High-dose regimen: 0.1–1 μM/L Low-dose regimen: <0.2 μM/L	Per protocol for determination of leucovorin rescue regimen	Decreased protein binding is observed in some situations, but implications for interpretation of total concentrations are unclear
Immunosuppressant Drugs			
Cyclosporine	100–500 mcg/L (whole blood, using specific assay)	Trough or 2-hr postdose; steady state may require up to 5 days	Highly variable unbound fraction in blood; higher total concentrations may be acceptable in patients with hypercholesterolemia or prior to acute rejection episodes (increased serum binding); lower total concentrations might be acceptable in patients with decreased binding in serum (low cholesterol)
Tacrolimus	Initiation: 20 mcg/L; Maintenance: 5–10 mcg/L	Trough concentrations three times a week initially until concentrations are stabilized; monitoring intervals can be extended with maintenance therapy	Therapeutic range may shift slightly with concomitant immunosuppressant medications and by indication; many drug interactions; consider impact on concentration when starting/stopping interacting medications
Psychotropics			
Lithium	0.5–1.2 mEq/L (acute management) 0.6–0.8 mEq/L (maintenance)	12 hr after the evening dose on BID or TID schedule; steady state may require up to 1 wk	Monovalent cation which is not bound to plasma proteins; does not undergo metabolism

AIDS = acquired immune deficiency syndrome.

SAMPLE TIMING

Incorrect timing of sample collection is the most frequent source of error when therapeutic drug monitoring results do not agree with the clinical picture.[24,36] Warner reviewed five studies in which 70–86% of the samples obtained for therapeutic drug monitoring purposes were not usable. In most cases, this was the result of inappropriate sample timing, including lack of attention to the time required to reach a steady state.[24] There are two primary considerations for sample timing: (1) how long to wait after initiation or adjustment of a dosage regimen, and (2) when to obtain the sample during a dosing interval.

At Steady State

When a drug dosage regimen (a fixed dose given at a regularly repeated interval) is initiated, concentrations are initially low and gradually increase until a steady state is reached. Pharmacokinetically, *steady state* is defined as the condition in which the rate of drug entering the body is equal to the rate of its elimination. For the purpose of therapeutic drug monitoring, a steady state means that drug concentrations have leveled off at their highest and, when given as the same dose at a fixed interval, the concentration versus time profiles are constant from interval to interval. This is illustrated in **Figure 6-3** for a constant infusion and a chronic intermittent dosage regimen.

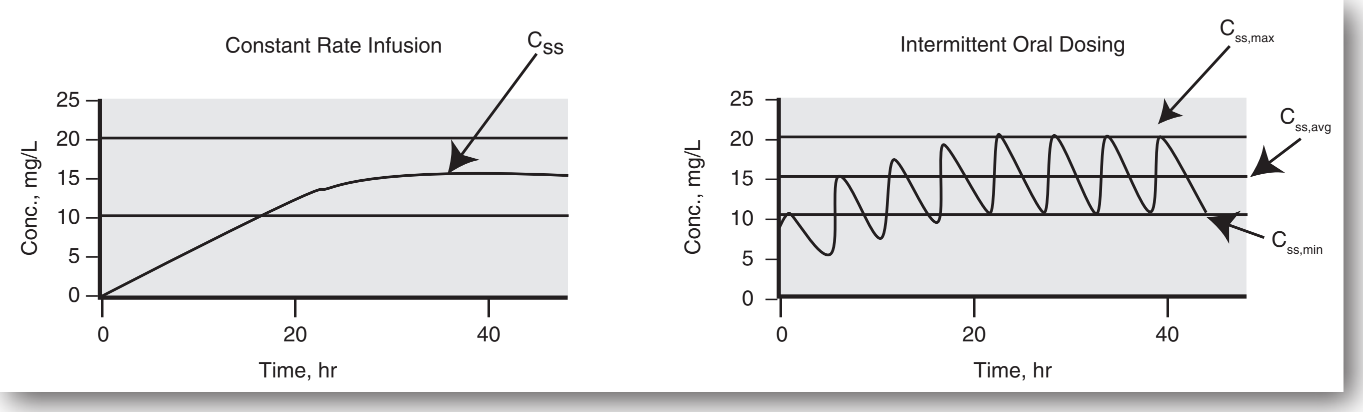

FIGURE 6-3. Concentration versus time plots for a constant infusion and intermittent therapy after initiation of therapy, without a loading dose. The half-life for this hypothetical drug is eight hours. Thus, 88% of the eventual average steady-state concentration ($C_{ss,avg}$) is attained in 24 hours.

Drug concentration measurements should not be made until the drug is sufficiently close to a steady state, so that the maximum benefit of the drug is ensured. The time required to reach a steady state can be predicted if the drug's half-life is known, as follows:

NUMBER OF HALF-LIVES	PERCENTAGE OF STEADY STATE ATTAINED
2	75%
3	88%
4	94%
5	97%

This means the clinician should wait three half-lives at a minimum before obtaining a sample for monitoring purposes. The clinician also should anticipate that the "usual" half-life in a given patient may be actually longer due to impaired elimination processes, and it may be prudent to wait longer if possible. The half-lives of drugs that are typically monitored are reported in the Applications section and typical times to steady state are reported in Table 6-3.

Sometimes drugs are not given as a fixed dose at a fixed interval, or they may undergo diurnal variations in pharmacokinetic handling.[37,38] Although the concentration-versus-time profiles may differ from each other within a given day, the patterns from day-to-day will be the same if a steady state has been attained. In cases of irregular dosing or diurnal variations, it is important that drug concentration measurements on different visits be obtained at similar times of the day for comparative purposes.

An unusual situation is caused by autoinduction, as exemplified by carbamazepine. The half-life of carbamazepine is longer after the first dose but progressively shortens as the enzymes that metabolize carbamazepine are induced by exposure to itself.[39] The half-life of carbamazepine during chronic therapy cannot be used to predict the time required to reach a steady state. The actual time to reach a steady state is somewhere between the time based on the first-dose half-life and that based on the chronic-dosing half-life. For this reason, patients are typically started on 25–33% of the target total daily dose.[40] Autoinduction has been found to be reversible when carbamazepine is held for six or more days.[41]

It is a common misconception that a steady state is reached faster when a loading dose is given. Although a carefully chosen loading dose will provide desired target concentrations following that first dose, the resulting concentration is only an approximation of the true steady-state concentration, and it will still require at least three half-lives to attain a true steady state. Whenever possible, it is best to allow more time for a steady state to be attained than less. This is also important because the average half-life for the population may not apply to a specific patient.

There are some exceptions to the rule of waiting until a steady state is reached before sampling. If there is suspected toxicity early during therapy, a drug concentration measurement is warranted and may necessitate immediate reduction or suspension of a dose or dose rate. Dosing methods designed to predict maintenance dosage regimens using pre-steady-state drug concentrations are useful when rapid individualization of the dosage regimen is needed.[42-47]

Within the Dosing Interval

Figure 6-3 shows typical concentration versus time profiles for a drug given by constant infusion and a drug given by oral intermittent dosing. Once a steady state is attained, drug concentrations during a constant infusion remain constant, and samples for drug concentration measurements can be obtained at any time. When a drug is given intermittently, however, there is fluctuation in the drug concentration profile. The lowest concentration during the interval is known as the *steady-state minimum concentration*, or the *trough*. The highest concentration is known as the *steady-state maximum concentration*, or the *peak*. Also shown in Figure 6-3 is the *steady-state average concentration* ($C_{ss,avg}$), which represents the time-averaged concentration during the dosage interval. An important principle of dosing for drugs that show first-order behavior is that the average concentration during the interval or day will change in direct proportion to the change in the daily dose. This is covered in more detail in the Use of Concentrations for Dosage Adjustment section.

The degree of fluctuation within a dosing interval will depend on three factors: the half-life of the drug in that patient; how quickly the drug is absorbed (as reflected by the time at which a peak concentration occurs for that particular formulation); and the dosing interval. The least fluctuation (lowest peak:trough ratio) will occur for drugs with relatively long half-lives that are slowly absorbed or given as sustained-release formulations (prolonged peak time) in divided doses (short dosing interval). However, drugs with relatively short half-lives that are quickly absorbed (or given as prompt-release products) and given only once daily will show the greatest amount of fluctuation within the interval.

The estimated degree of fluctuation (peak:trough ratio) for a given dosage regimen can be estimated by comparing the drug's half-life in a patient to the difference between the dosing interval and the estimated time required to reach a peak concentration.[48] The following guidelines may be used:

(INTERVAL–PEAK TIME)/ HALF-LIFE	PEAK:TROUGH RATIO
2.00	4.0
1.50	2.8
1.00	2.0
0.50	1.4
0.25	1.2

Using the information above, concentrations during the interval fluctuate very little (peaks are only 1.2 times troughs) if the difference between the dosing interval and peak time is one quarter of the drug's half-life. In that case, it may be assumed that concentrations obtained anytime during the interval are almost equivalent; the peak, trough, and average concentrations are roughly equal.

The choice of timing for samples within the dosing interval should be based on the clinical question to be addressed. Troughs are usually recommended for therapeutic confirmation, especially if the therapeutic range was formulated based on trough concentrations as for most of the antiepileptic drugs.[15] Trough concentrations are also recommended if the indication for concentration monitoring is avoidance of inefficacy or distinguishing nonadherence from therapeutic failure. Trough concentrations should also be monitored if the patient tends to experience symptoms of inefficacy before the next dose (in which case a shortening of the dosing interval might be all that is needed). Although it is logical to assume that the lowest concentration during the interval will occur immediately before the next dose, this is not always the case. Some products are formulated as delayed-release products (e.g., enteric-coated valproic acid [VPA]) that are designed to be absorbed from the intestine rather than the stomach. As such, they may not begin to be absorbed for several hours after administration, and the concentration of drug from the previous dose continues to decline for several hours into the next interval. It is important to recognize that the predose concentration for those formulations is not the lowest concentration during the interval (**Figure 6-4**).

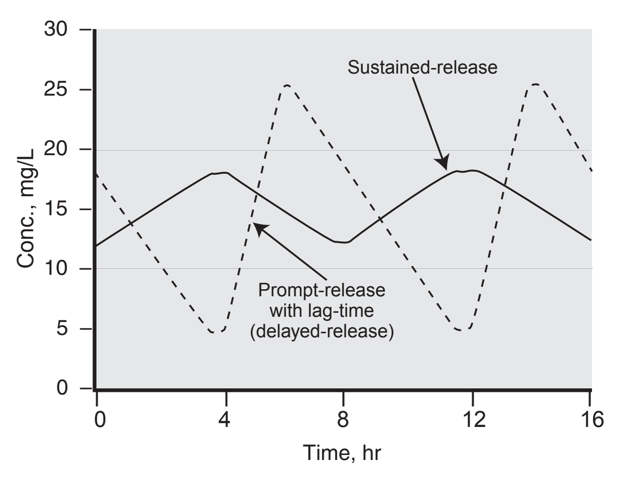

FIGURE 6-4. Concentration versus time profiles for a prompt-release formulation that exhibits a lag-time in its release or absorption (delayed-release) as compared to a sustained-release formulation without lag-time. Note that the lowest concentration during the dosing interval for the delayed-release product occurs at a time that is typically expected for the peak to occur.

Peak concentrations are monitored less often for drugs given orally because the time at which peak concentrations occur is difficult to predict. If a peak concentration is indicated, the package insert should be consulted for peak times of individual products. Peak concentration monitoring would be appropriate if the patient complains of symptoms of toxicity at a time believed to correspond with a peak concentration. Peaks may also be used for intravenous (IV) drugs (e.g., aminoglycosides) because the time of the peak is known to correspond to the end of the infusion. For the aminoglycosides, the peak concentration is believed to be a predictor of efficacy.[49]

Sometimes the clinician wishes to get an idea of the average concentration of drug during the day or dosing interval. This is particularly useful when the concentration is to be used for a dosage adjustment. Pharmacokinetically, the average concentration equals the area under the curve (AUC) during the dosing interval (requiring multiple samples) divided by the interval. (Note: The AUC during an interval or portion of an interval is used as the monitoring parameter in place of single drug concentration measurements for certain drugs, such as some immunosuppressant and cytotoxic drugs, because it provides a better indication of overall drug exposure.) However, determination of the AUC, or $C_{ss,avg}$, by multiple sampling is not cost-effective for the most commonly monitored drugs. The following are alternatives to estimating the $C_{ss,avg}$ without multiple samples:

- Look up the expected time to reach a peak concentration for the particular formulation and obtain a sample midway between that time and the end of the dosing interval.[25]

Measure the trough concentration (as close to the time of administration of the next dose as possible) and use that along with the population value for the drug's volume of distribution (V_d) to estimate the peak concentration as shown below. Then take the average of the trough and the peak to get an estimate of the average steady-state concentration.

$$\text{Peak}_{\text{steady-state}} = \left(\text{Dose}/\ V_d\right) + \text{measured trough}$$

- If you have reason to believe that there is very little fluctuation during the dosing interval, then a sample drawn anytime during the interval will provide a reasonable reflection of the average concentration.

There are special and extremely important timing considerations for some drugs, such as digoxin. It must reach specific receptors, presumably in the myocardium, to exhibit its therapeutic effect, but this takes a number of hours after the dose is administered. Early after a digoxin dose, concentrations in serum are relatively high, but response is not yet evident because digoxin has not yet equilibrated at its site of action. Thus, only digoxin concentrations that are in the postdistribution phase should be monitored and compared to the reported therapeutic range (**Minicase 2**).

The timing of samples for other drugs may be based on the requirements for certain dosing methods. This is true for the aminoglycosides and certain lithium dosing methods. Sample timing for drugs like methotrexate will be specified in protocols because concentrations are used to determine the need for rescue therapy with leucovorin to minimize methotrexate toxicity.

Although samples for drug concentration measurements may be preferred at certain times during a dosing interval, visits to physician offices often do not coincide with desired times for blood draws. One is then faced with the matter of how to interpret a concentration that is drawn at a time that happens to be more convenient to the patient's appointment. The most critical pieces of information to obtain in this situation are (1) when the last drug dose was taken; (2) adherence; (3) timing of the sample relative to the last dose; and (4) the expected time of peak concentration. Some drugs are available as a wide variety of formulations (solutions, suspensions, prompt-release, and sustained-release or extended-release solid dosage forms), and the package insert may be the best source of information for the expected peak time. Once again, drugs with relatively long half-lives given as sustained-release or slowly absorbed products in divided doses will have the flattest concentration-versus-time profiles, and concentrations drawn anytime during

MINICASE 2

Importance of Sample Timing for Digoxin

Robert P., a 75-year-old male patient with heart failure, chronic kidney disease, and atrial fibrillation, is admitted to the hospital with hospital-acquired pneumonia. He was discharged two weeks ago following a heart failure exacerbation. Five days into his current stay in the intensive care unit (ICU), he becomes increasingly confused, and his family members voice concern that his confusion could be related to his medications. At this institution, all daily medications are administered at 0900 unless otherwise specified. The patient is also complaining of nausea. His medication regimen includes the following:

Home medications:
- Lisinopril 10 mg PO daily
- Metoprolol succinate 50 mg PO daily
- Digoxin 0.25 mg PO daily
- Spironolactone 25 mg PO daily
- Aspirin 81 mg PO daily
- Furosemide 40 mg PO BID
- Potassium chloride 20 mEq PO daily
- Warfarin 4 mg PO daily at 1800

Started on admission:
- Piperacillin–tazobactam 2.25g IV q 6 hr
- Ciprofloxacin 400 mg IV q 24 hr
- Methylprednisolone 40 mg IV daily

After reviewing his medication regimen, you suggest drawing a digoxin serum concentration due to multiple risk factors for toxicity (age, renal dysfunction, relatively higher dose, symptoms of toxicity). Robert P.'s digoxin concentration is drawn at 1200 and returns at 2.2 mcg/L; the medical resident suggests a decrease in dose to 0.125 mg PO daily. The desired range of serum digoxin concentrations for treatment of heart failure in Robert P. is 0.5–1 mcg/L.

QUESTIONS: What considerations should be taken into account in the interpretation of this patient's digoxin concentration? Are there other possible causes for his new onset confusion? What recommendation should be made at this time?

DISCUSSION: There are several nondrug-related reasons that may have resulted in this patient's confusion: ICU delirium, increased blood urea nitrogen due to renal dysfunction, electrolyte imbalances, corticosteroid administration, infection leading to altered mental status, or undiagnosed mental health condition. There are no known interactions with the new medications initiated during his stay; therefore, drug interactions may be ruled out as an explanation for the high digoxin concentration. Digoxin concentrations, with maintenance therapy, should be drawn at the trough of the therapeutic interval (just prior to administration of the next dose) or no sooner than six to eight hours after the dose was administered. His digoxin concentration in serum, however, was drawn three hours after his 0900 dose and, therefore, does not yet reflect the concentration of digoxin at its myocardial target. To determine if digoxin is the primary cause of his confusion, a repeat concentration should be drawn after 1500 today to account for the distribution of digoxin to tissues. Changes to the medication regimen should be based on the correctly drawn concentration.

the interval are going to be similar. However, prompt-release drugs with short half-lives given less frequently will show more fluctuation. Knowing the expected peak time for the formulation in question is especially important for drugs that show more fluctuation during that interval. In that case, one can at least judge if the reported concentration is closer to a peak, an average (if midway between the peak and trough), or a trough.

USE OF CONCENTRATIONS FOR DOSAGE ADJUSTMENT

A chronic intermittent dosage regimen has three components: the dose rate, the dosing interval, and the dose. For the dosage regimen of 240 mg q 8 hr, the dose is 240 mg, the interval is 8 hours, and the dose rate can be expressed as 720 mg/day or 30 mg/hr. The dose rate is important because it determines the average concentration ($C_{ss,avg}$) during the day. The degree of fluctuation within a dosing interval is highly influenced by the dosing interval.

Dosage Adjustments for Linear Behavior

If a drug is known to have first-order bioavailability and elimination behavior after therapeutic doses, one can use simple proportionality to make an adjustment in the daily dose:

- If average concentrations are being monitored or estimated, one can predict that the average, steady-state drug concentration will increase in proportion to the increase in daily dose, regardless of any changes that were made in the dosing interval.
- If trough concentrations are monitored and the dosing interval will be held constant, the trough concentration will increase in proportion to the increase in daily dose.
- If trough concentrations are monitored for a drug that exhibits considerable fluctuation during the interval and both the dose rate and dosing interval will be adjusted, the trough concentration will not be as easy to predict at a new steady state and is beyond the scope of this chapter. If the trough concentration can be used to estimate the $C_{ss,avg}$, as described above, the $C_{ss,avg}$ can be predicted with certainty to change in proportion to the change in daily dose.

Sampling after a dosage regimen adjustment, if appropriate, should not be done until a new steady state has been reached. For a drug with first-order behavior, this should take the same period of time (three half-lives at a minimum) that it did after initiation of therapy with this drug.

Dosage Adjustments for Drugs with Nonlinear Behavior

All drugs will show nonlinear elimination behavior if sufficiently high doses are given. Some drugs, however, show pronounced nonlinear (Michaelis-Menten) elimination behavior following doses that produce therapeutic drug concentrations. This means that an increase in the dose rate of the drug will result in a greater-than-proportional increase in the drug concentration. Phenytoin is an example of a drug with this behavior. Theophylline and procainamide also show some degree of nonlinear behavior but only at the higher end of their therapeutic ranges (and not enough to require special dosing methods).

Methods have been described to permit predictions of the effect of dose rate increases for phenytoin using population averages or actual measurements of the parameters that define nonlinearity, namely V_{max} (maximum rate of metabolism) and K_m (the "Michaelis constant"), but they are beyond the scope of this chapter.[50] The most important rule to remember for dosage adjustments of drugs like phenytoin is to be conservative; small increases in the dose rate will produce unpredictably large increases in the serum drug concentration. It must also be remembered that the half-life of a drug like phenytoin will be progressively prolonged at higher dose rates. Increases in dose rate will require a longer period of time to reach a steady state as compared to when the drug was first initiated.

Population pharmacokinetic or Bayesian dosage adjustment methods, which involve the use of statistical probabilities, are preferred by many for computerized individualization of therapy.[47,51] They are useful for drugs with both linear and nonlinear behavior.

PROTEIN BINDING, ACTIVE METABOLITES, AND OTHER CONSIDERATIONS

Altered Serum Binding

Total (unbound plus protein-bound) drug concentrations measured in blood, serum, or plasma are almost always used for therapeutic drug monitoring, despite the fact that unbound drug concentrations are more closely correlated to drug effect.[35] This is because it is easier to measure the total concentration and because the ratio of unbound to total drug concentration in serum is usually constant within and between individuals. For some drugs, however, the relationship between unbound and total drug concentration is extremely variable among patients, or it may be altered by disease or drug interactions. For drugs that undergo concentration-dependent serum binding, the relationship between unbound and total concentration varies within patients. In all of these situations, total drug concentration does not reflect the same concentration of activity as with normal binding and must be cautiously interpreted because the usual therapeutic range will not apply (**Minicase 3**).

The direct measurement of unbound drug concentration would seem to be appropriate in these situations. Drugs for which total concentration monitoring is routinely performed (but for which unbound concentration monitoring has been proposed) include carbamazepine, phenytoin, and VPA. Of these, correlations between unbound drug concentration and response have been weakly established for carbamazepine but more firmly established for phenytoin.[35,53]

Unbound drug concentration measurements involve an extra step prior to analysis—separation of the unbound from the bound drug. If unbound drug concentration measurements are unavailable, too costly, or considered impractical, the following

MINICASE 3

Value of Unbound Antiepileptic Drug Serum Concentrations

Frank S., a 66-year-old Caucasian male, is admitted to the emergency department (ED) with a chief complaint of vomiting, diarrhea, blurred vision, and unsteady gait. He was diagnosed with epilepsy three years ago and is taking the following oral antiepileptic drugs at home: VPA 1000 mg BID; sodium phenytoin 200 mg TID; carbamazepine 300 mg BID; and levetiracetam 400 mg at bedtime. The patient also has a past medical history of hypertension and hyperlipidemia and is taking aspirin 81 mg every day, simvastatin 40 mg at bedtime, and metoprolol 100 mg BID. Physical examination in the ED reveals bilateral nystagmus and significant ataxia. The clinical picture is deemed consistent with antiepileptic drug toxicity and total serum concentrations of three of the antiepileptic drugs that are ordered:

Carbamazepine: 6.4 mg/L (reference range 4–12 mg/L)
Phenytoin: 9.3 mg/L (reference range 10–20 mg/L)
VPA: 72 mg/L (reference range 50–100 mg/L)

Free concentrations of the same three drugs are subsequently determined:

Carbamazepine: 1.9 mg/L (reference range 1–3 mg/L)
Phenytoin: 1.3 mg/L (reference range 1–2 mg/L)
VPA: 13.4 mg/L (reference range 2.5–10 mg/L)
Serum albumin: 4 g/dL

The VPA is held for 24 hours and then reintroduced at a dose rate of 250 mg TID. His symptoms resolve within 24 hours. A repeat unbound serum VPA concentration one week later is 5.2 mg/L.

QUESTIONS: How were total concentrations of antiepileptic drugs misleading in this patient? How might sole reliance on total antiepileptic serum concentrations have led to a different clinical decision and outcome? How did free serum concentration monitoring aid in understanding the cause of the patient's signs and symptoms?

DISCUSSION: This patient has total serum concentrations of carbamazepine and VPA within the reference ranges for total concentrations of these drugs, while the total concentration of phenytoin is slightly below the lower limit of the laboratory's reference range. Based solely on these total drug concentrations, the unaware clinician would be tempted to seek alternative explanations and likely delay the resolution of the patient's signs and symptoms. Even worse, the clinician might be tempted to increase the daily dose of sodium phenytoin in an attempt to get the total phenytoin (PHT) concentration to within the usual reference range for phenytoin. Measurement of free concentrations of these antiepileptics reveals that the patient is probably getting appropriate daily doses of carbamazepine and phenytoin but is clearly receiving too much VPA. This is confirmed when the signs and symptoms resolve after reduction of the VPA dose rate and a repeat unbound serum VPA concentration at a new steady state is within the laboratory's reference range for free VPA.

Explanations for the *supratherapeutic* free VPA concentration in face of a *therapeutic* total concentration in this patient may include one or more of the following: (1) saturable (nonlinear) protein binding of VPA to albumin at higher dose rates of VPA; (2) inhibition of VPA metabolism by salicylic acid; and (3) displacement of VPA from albumin by phenytoin and salicylic acid. As a result, total VPA concentrations no longer reflect what is happening to the free, active VPA moiety, and the usual therapeutic reference range of total concentrations cannot be used.

(Minicase 3 is adapted from reference 52.)

alternative approaches to interpreting total drug concentrations in situations of altered serum protein binding may be used:

- **Use of equations to normalize the measured total concentration**—Sheiner and Tozer were the first to propose equations that can be used to convert a measured total concentration of drug (phenytoin in this case) to an approximation of what the total concentration would be if the patient had normal binding.[54] Equations to normalize PHT concentrations have been used for patients with hypoalbuminemia, impaired renal function, and concurrent VPA therapy.[55] Once the total concentration has been normalized, it may be compared to the conventional therapeutic range. It must be noted that this normalization method may not be a reliable substitute for measurement of the unbound phenytoin concentration.
- **Normalize the measured total concentration using literature estimates of the abnormal unbound drug fraction**—An alternative method for normalizing the total concentration can be used if reasonable estimates of the abnormal and normal unbound reactions of the drug can be ascertained (i.e., from the literature). The normalized total concentration ($C_{\text{normalized}}$) can be estimated as

$$C_{\text{normalized}} = C_{\text{measured}} \times \frac{\text{abnormal unbound fraction}}{\text{normal unbound fraction}}$$

  where C_{measured} is the measured total concentration reported by the laboratory.
- **Predictive linear regression equations**—Some studies have reported the ability to predict unbound drug concentrations in the presence of displacing drugs if the total concentrations of both drugs are known. This has been done to predict unbound concentrations of phenytoin and carbamazepine, both in the presence of VPA.[56,57] These unbound drug concentrations should be compared to corresponding therapeutic ranges of unbound drug, which can be estimated for any drug if the normal unbound fraction and the usual therapeutic range of total concentrations (TR) are known:

$$TR_u = TR \times \text{normal unbound fraction}$$

- **Use of saliva as a substitute for unbound drug concentration**—This may be a reasonable alternative so long as studies have shown a strong correlation between unbound concentrations in serum and concentrations in saliva. The concentration of drug in saliva may not be equal to the concentration in serum ultrafiltrate. Therefore, the laboratory should have determined a reliable conversion factor for this. The calculated unbound concentration may then be compared to the estimated therapeutic range for unbound concentrations as described above. Saliva concentrations may also be used as a predictor of total drug concentrations, particularly for some of the antiepileptic drugs.[58]
- **Active metabolites**—Interpretation of parent drug concentration alone, for drugs with active metabolites that are present to varying extents, is difficult at best. Active metabolites may contribute to therapeutic response, to toxicity, or to both. Because metabolites will likely have different pharmacokinetic characteristics, they will be affected differently than the parent drug under different physiologic and pathologic conditions. For drugs like primidone (metabolized to phenobarbital) and procainamide (metabolized to N-acetylprocainamide [NAPA]), the laboratory will typically report both the parent drug and the metabolite as well as a therapeutic range for both. Although a therapeutic range for the sum of procainamide and NAPA may be reported by some laboratories, this practice is discouraged because the parent and metabolites have different types of pharmacologic activities.
- **Enantiomeric pairs**—Some drugs exist as an equal mixture (racemic mixture) of enantiomers, which are chemically identical but are mirror images of each other. Because they can interact differently with receptors, they may have very different pharmacodynamic and pharmacokinetic properties. The relative proportions of the enantiomers can differ widely among and within patients. Thus, a given concentration of the summed enantiomers (what is routinely measured using achiral methods) can represent very different activities.[31]

Table 6-3 provides relevant information about protein binding, active metabolites, and other influences on serum concentration interpretation for drugs discussed in the Applications sections that follow.

APPLICATIONS

Analgesic Drugs

Acetaminophen

Therapeutic range and clinical considerations. Acetaminophen is the first-line treatment for patients with osteoarthritis and mild-to-moderate chronic pain.[59,60] Additionally, acetaminophen is a commonly used antipyretic agent.[61] Acetaminophen serum concentrations should be monitored only when suspected or confirmed overdose is a concern or in patients taking long-term acetaminophen with concomitant liver dysfunction.[62] Antipyretic activity is expected to occur at serum concentrations of 4–18 mg/L; analgesic activity is expected to occur at serum concentrations of 10 mg/L.[63] Historically, 4000 mg/day has long been considered a safe therapeutic dose in normal patients; however, in 2009 the FDA published a recommendation to lower the maximum daily dose to 3250 mg/day.[64-66]

Sample timing. Acetaminophen serum concentrations should be drawn at least four hours after suspected acute ingestion; the Rumack–Matthew nomogram should be referenced to determine treatment decisions.[67] In the case of IV formulation overdose, reliable guidelines related to monitoring and management have not been established.

Protein binding, active metabolites, and other considerations. Acetaminophen is 10–25% bound to protein at therapeutic concentrations and 8–43% at supratherapeutic concentrations.[61]

Bronchodilators

Aminophylline and Theophylline

Therapeutic range and clinical considerations. Although aminophylline and theophylline are rarely used in practice, serum concentration monitoring may be necessary because of variable pharmacokinetics. Aminophylline is used as a second-line option in the management of chronic obstructive pulmonary (COPD) exacerbations if short-acting β-agonists are found to be ineffective.[68-72] In contrast, it is not recommended for the treatment of asthma exacerbations according to the Global Initiative for Asthma (GINA) 2015 guidelines.[73,74] Peak concentrations of 5–15 mg/L are targeted with concentrations >20 mg/L being associated with adverse events.[75] The GINA 2015 guidelines do not recommend the use of theophylline in children <12 years old, and nonsustained release formulations should be considered only for adults as an alternative therapy to more effective inhaled corticosteroids.[73,76-78] The 2017 Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines recommend using a more effective therapy of inhaled long-acting bronchodilators over theophylline when possible.[79] In the management of COPD exacerbations, IV theophylline can be considered as a second-line option if short-acting β-agonists are ineffective.[68-72] In contrast, theophylline is not recommended in the treatment of asthma exacerbations.[80] There is an 85% probability of adverse effects with concentrations above 25 mg/L; concentrations above 30–40 mg/L are associated with dangerous adverse events.[81] Many patients do not tolerate theophylline; adverse effects typically experienced by adults include nausea, vomiting, diarrhea, irritability, and insomnia at concentrations above 15 mg/L; supraventricular tachycardia, hypotension, and ventricular arrhythmias at concentrations above 40 mg/L; and seizures, brain damage, and even death at higher concentrations.[82]

Theophylline is also indicated for treatment of neonatal apnea, although caffeine is preferred.[83] The therapeutic range of theophylline in neonates is generally considered to be 5–10 mg/L but may be as low as 3 mg/L or as high as 14 mg/L.[84-87] Adverse effects in neonates include lack of weight gain, sleeplessness, irritability, diuresis, dehydration, hyperreflexia, jitteriness, and serious cardiovascular and neurologic events.[81] Tachycardia has been reported in neonates with concentrations as low as 13 mg/L.[88]

In summary, there is considerable overlap of therapeutic and toxic effects within the usual therapeutic ranges reported for theophylline in neonates, children, and adults. Indications for theophylline monitoring include therapeutic confirmation of effective concentrations after initiation of therapy or a dosage regimen adjustment, anticipated drug–drug interactions, change in smoking habits, and changes in health status that might affect the metabolism of theophylline.

Sample timing. Concentrations should be measured 30 minutes after the end of the infusion if an aminophylline loading dose is given; continuous infusion monitoring should include monitoring serum concentrations at one half-life after the start of the infusion and at every 12 or 24 hours after the start of the infusion.[89] The half-life of theophylline can range anywhere from 20–30 hours in premature infants to three to five hours in children or adult smokers to as long as 50 hours in nonsmoking adults with severe heart failure or liver disease.[75,84] Steady state will be reached in 24 hours for the average patient with an elimination half-life of eight hours but will require a much longer elimination time for patients with heart failure or liver disease. The time to steady state in premature neonates may be as long as nine days.[85]

The fluctuation of theophylline concentrations within a steady-state dosing interval can be quite variable—depending not only on the frequency of administration, but also on the type of formulation, half-life, and whether or not the dose was taken with a meal.[81,82] Trough concentrations of theophylline are most reproducible and should always be obtained. Comparisons of trough concentrations from visit to visit will also be facilitated if samples are obtained at the same time of day on each visit. This is because of diurnal variations in the rate of theophylline absorption.[81]

Use of concentrations for dosage adjustment. Theophylline is usually assumed to undergo first-order elimination, but some of its metabolic pathways are nonlinear at concentrations at the higher end of the therapeutic range.[81] The clearance of theophylline decreases by 20% as daily doses are increased from 210 mg to 1260 mg.[81] For IV formulations, concentrations should be checked every 24 hours to determine if dose adjustment is warranted; for oral formulations intervals of 6–12 months are recommended once the daily dose has been stabilized.[75,90]

Protein binding, active metabolites, and other considerations. Theophylline is 35% bound to serum proteins in neonates and 40–50% bound to serum proteins in adults. Therefore, significant alterations in serum protein binding are unlikely.[81] Theophylline is metabolized to the active metabolite caffeine, which is of minor consequence in adults. Caffeine concentrations in the serum of neonates, however, are approximately 30% of theophylline concentrations and contribute to treatment of neonatal apnea. This may account for the slightly lower therapeutic range of theophylline in neonates as compared to adults.

Caffeine

Therapeutic range and clinical considerations. Caffeine is indicated for neonatal apnea (apnea of prematurity) and is recommended over theophylline because it can be given once daily and is considered to have a wider therapeutic range.[83] Concentrations as low as 5 mg/L may be effective, but most pediatric textbooks consider 10 mg/L to be the lower limit of the therapeutic range; an acceptable reference range is 8–14 mg/L.[87,91,92] Toxicity may occur at concentrations >20 mg/L, and serious toxicity is associated with serum concentrations above 50 mg/L. Signs of toxicity include jitteriness, vomiting, irritability, tremor of the extremities, tachypnea, and tonic-clonic movements. Serum concentration measurements of caffeine may not be routinely necessary for apnea of prematurity in neonates.[93] Neonates who do not respond as expected or in whom there is recurrence of apnea after a favorable response may benefit, however.

Sample timing. The half-life of caffeine in preterm infants at birth ranges from 40–230 hours.[91,92] Thus, a loading dose is always administered to attain effective concentrations as soon as possible. The long half-life means that caffeine concentrations will not fluctuate much during the interval, even when caffeine is administered once daily. Sampling in the postdistribution phase is recommended, but at least 2 hours postdose.

Baseline concentrations of caffeine must be obtained prior to the first caffeine dose in the following situations: (1) if the infant had been previously treated with theophylline because caffeine is a metabolite of theophylline; and (2) if the infant was born to a mother who consumed caffeine prior to delivery. Reductions in the usual caffeine dose will be necessary if predose caffeine concentrations are present.

Use of concentrations for dosage adjustment. There are no data to suggest that caffeine undergoes nonlinear elimination. Thus, dosage adjustments by proportionality are acceptable. Dosage adjustments for caffeine are complicated by the fact that a true steady state is not reached for at least four days, so any adjustments should be conservative.

Protein binding, active metabolites, and other considerations. Caffeine is only 31% bound to serum proteins and has no active metabolites.[91]

Antiepileptics

The antiepileptics that have clearly defined therapeutic ranges should be routinely monitored. Because they are used as prophylaxis for seizures that may not occur frequently, it is particularly important that effective serum concentrations of these drugs be ensured early in therapy. Indications for monitoring antiepileptic drugs include[15,94]: (1) documentation of an effective steady-state concentration after initiation of therapy; (2) after dosage regimen adjustments; (3) after adding a drug that has potential for interaction; (4) changes in disease state or physiologic status that may affect the pharmacokinetics of the drug; (5) within hours of a seizure recurrence; (6) after an unexplained change in seizure frequency; (7) suspected dose-related drug toxicity; and (8) suspected nonadherence.

Carbamazepine

Therapeutic range and clinical considerations. Carbamazepine is indicated for the prevention of partial seizures and generalized tonic-clonic seizures, and the treatment of pain associated with trigeminal neuralgia.[84,95] Extended-release formulations have been approved for the prevention and treatment of acute manic or mixed episodes in patients with bipolar I disorder.[96] Most textbooks report a therapeutic range of 4–12 mg/L. Concentrations above 12 mg/L are most often associated with nausea and vomiting, unsteadiness, blurred vision, drowsiness, dizziness, and headaches in patients taking carbamazepine alone.[39] Patients taking other antiepileptic drugs, such as primidone, phenobarbital, VPA, or phenytoin, may show similar adverse effects at concentrations as low as 9 mg/L. For this reason, many clinicians use a more conservative target therapeutic range of 4–8 mg/L.[95] Toxicity is expected at concentrations >20 mg/L; serious adverse reactions are seen at concentrations >50 mg/L.[97,98] Some central nervous system (CNS) effects such as drowsiness, dizziness, or headaches can be seen at concentrations >8 mg/L.[99] Some clinicians target the lower end of the therapeutic range (4–8 mg/L) to avoid adverse effects seen at concentrations of 11–15 mg/L (somnolence, nystagmus, ataxia) or toxicities at 15–25 mg/L of agitation, hallucinations, and chorea.[94]

Carbamazepine 10,11-epoxide is an active metabolite that can be present in concentrations containing 12–25% carbamazepine, but it is not routinely monitored along with the parent drug. A suggested therapeutic range for this metabolite, used at some research centers, is 0.4–4 mg/L; toxicity is expected at concentration >9 mg/L. [84,100]

In addition to the usual indications for monitoring, it is important to monitor carbamazepine concentrations if the patient is switched to another formulation (e.g., generic), as bioavailability may vary between formulations.[94]

Sample timing. If a loading dose is administered, concentrations can be taken after two hours of administration of the suspension formulation to ensure therapeutic concentrations have been achieved.[101] Because carbamazepine induces its own metabolism, it is recommended that initial dose rates of carbamazepine be relatively low and gradually increased over a three-to-four week period.[39] For maximal induction or deinduction to occur, two to three weeks may be required after the maximum dose rate has been attained. Thus, a total of six to seven weeks may be required for a true steady state to be reached after initiation of therapy. After any dose rate changes or addition/discontinuation of enzyme-inducing or inhibiting drugs, two to three weeks will be required to reach a new steady state.[84] It is recommended that during dose titration carbamazepine concentrations should be measured at weekly intervals. After reaching the maintenance dose, blood concentrations can be less frequently monitored at three-to-six month intervals.[101]

A trough concentration is generally preferred. The absorption of immediate-release carbamazepine tablets from the gastrointestinal tract is relatively slow and erratic, reaching a peak between three and eight hours after a dose.[102] Extended-release formulations are even more slowly absorbed. If carbamazepine is administered every six or eight hours, serum concentrations during the dosing interval will remain fairly flat, and all concentrations will be fairly representative of a trough concentration. Less frequent dosing will result in more fluctuation in which case the time of the concentration relative to the last dose should be documented for appropriate interpretation. Use of the extended-release formulation of carbamazepine will minimize fluctuations caused by diurnal variations.[103] Nevertheless, it is recommended that samples on repeated visits always be obtained at the same time of the day for purposes of comparison.[94]

Use of concentrations for dosage adjustment. Carbamazepine exhibits first-order behavior following therapeutic doses. Thus, increases in dose rate will result in a proportional increase in the average steady-state concentration of carbamazepine. An additional 10–20% should be added or subtracted for a dose increase or dose decrease, respectively.[104] This additional 10–20% will account for autoinduction previously described. If the dose is adjusted without a change in the interval, a concentration drawn at the same time within the interval will increase in proportion to the increase in dose.

Protein binding, active metabolites, and other considerations. In most patients, carbamazepine is 70–80% bound to serum proteins, including albumin and α-1-acid glycoprotein (AAG).[105] In some patients, however, unbound percentages as low as 10% have been reported.[84] Measurements of unbound carbamazepine concentrations are not generally recommended or necessary. Total concentrations should be carefully interpreted in situations of suspected altered protein binding. Decreased binding might be anticipated in uremia, liver disease, hypoalbuminemia, or hyperbilirubinemia.[84] Increased binding might be rarely expected in cases of physiologic trauma due to elevated AAG concentrations. Because VPA has been shown to displace carbamazepine from albumin, an equation was proposed to predict unbound carbamazepine concentrations in this situation.[57] Correlations between saliva and unbound carbamazepine concentrations are strong.[106] Thus, saliva sampling might be considered in situations of suspected alterations in carbamazepine binding.[107]

Drug–drug interactions that are expected to result in a higher proportion of active 10,11-epoxide metabolite relative to the parent drug (e.g., concurrent phenytoin, phenobarbital, or VPA) may alter the activity associated with a given carbamazepine concentration. It is suggested that a lower therapeutic range of 4–8 mg/L be used when those drugs are given concurrently.[15] Carbamazepine-10, 11-epoxide is 50% protein bound.[108]

Phenobarbital/Primidone

Primidone and phenobarbital are both used for management of generalized tonic-clonic and partial seizures.[84] Phenobarbital is also used for febrile seizures in neonates and infants, while primidone is used for treatment of essential tremor in the elderly.[94,109] Although primidone has activity of its own, most clinicians believe that phenobarbital—a metabolite of primidone—is predominantly responsible for primidone's therapeutic effects.

Therapeutic ranges and clinical considerations. The therapeutic range of phenobarbital for treatment of tonic-clonic, febrile, and hypoxic ischemic seizures is generally regarded as 10–40 mg/L, while concentrations as high as 70 mg/L may be required for refractory status epilepticus.[94,109] Eighty-four percent of patients are likely to respond with concentrations between 10 and 40 mg/L.[109] Management of partial seizures seems to require higher phenobarbital concentrations than management of bilateral tonic-clonic seizures.[15] Concentrations of phenobarbital are always reported when primidone concentrations are ordered. The therapeutic range of primidone reported by most laboratories is 5–12 mg/L.[15,94] Fifteen to 20% of a primidone dose is metabolized to the active phenobarbital; the side effects of primidone are mostly related to phenobarbital.[84] Central nervous system side effects such as sedation and ataxia generally occur in chronically treated patients at phenobarbital concentrations between 35 and 80 mg/L. Stupor and coma have been reported at phenobarbital concentrations above 65 mg/L.[102] Clinicians should consider drawing concentrations when nonadherence or toxicity is suspected, if the patient is experiencing lack of efficacy, in patients with severe liver or kidney disease, in patients requiring dialysis, and when interacting medications are initiated or discontinued.[110]

Sample timing. The half-life of phenobarbital is the rate-limiting step for determining the time to reach steady state after primidone administration. The half-life of phenobarbital averages 5 days for neonates and four days for adults.[109] Because phenobarbital or primidone dosage may be initiated gradually, steady state is not attained until two to three weeks after full dosage has been implemented. Due to phenobarbital's long half-life, concentrations obtained anytime during the day would provide reasonable estimates of a trough concentration. Ideally, concentrations should be obtained from visit to visit at similar times of the day.[109] If IV phenobarbital is administered, serum concentrations should be drawn at least one hour postinfusion.[111]

Use of concentrations for dosage adjustment. Phenobarbital and primidone exhibit first-order elimination behavior; thus, a change in the dose rate of either drug will result in a proportional change in the average, steady-state serum concentrations.[102,109]

Protein binding, active metabolites, and other considerations. Phenobarbital is approximately 50% bound to serum proteins (albumin) in adults; primidone is not bound to serum proteins.[94,112] Thus, total concentrations of both drugs are reliable indicators of the active, unbound concentrations of these drugs. Although primidone has an active metabolite, phenylethylmalonamide (PEMA), its contribution to activity is unlikely to be significant. Clearance of phenobarbital can increase or decrease depending on many patient-specific factors including age (decreases in neonates and elderly patients), severe liver or kidney disease (decreases), interacting medications (increases or decreases), urine pH (increases), and malnutrition (increases).[113-119]

Phenytoin and Fosphenytoin

Therapeutic range. Phenytoin is primarily used for treatment of generalized tonic-clonic and complex partial seizures.[120] It may also be used in the treatment of trigeminal neuralgia and for seizure prophylaxis following neurosurgery.[84,120] Studies have shown that serum concentrations of phenytoin between 10 and 20 mg/L will result in maximum protection from primary or secondary generalized tonic-clonic seizures in most adult patients with normal serum binding. Ten percent of patients with controlled seizures have phenytoin concentrations <3 mg/L, 50% have concentrations <7 mg/L, and 90% have concentrations <15 mg/L.[15] Concentrations at the lower end of the range are effective for bilateral seizures, while higher concentrations appear to be necessary for partial seizures.[15] The therapeutic range of total concentrations in infants is lower due to lower serum protein binding: 6–11 mg/L.[94] Concentration-related side effects include nystagmus; central nervous system depression (ataxia, inability to concentrate, confusion, and drowsiness); and changes in mental status, coma, or seizures at concentrations above 40 mg/L.[94] Although mild side effects may be observed at concentrations as low as 5 mg/L, there have been cases in which concentrations as high as 50 mg/L have been required for effective treatment without negative consequences.[121]

Some clinicians have proposed that monitoring of phenytoin be limited to unbound concentrations, particularly in patients who are critically ill or likely to have unusual protein binding.[53,122,123] Unbound phenytoin concentrations are more predictive of clinical toxicity than are PHT concentrations in these individuals.[124] The therapeutic range of unbound phenytoin concentrations is presumed to be 1–2 mg/L for laboratories that determine the unbound phenytoin fraction at 25 °C, and 1.5–3 mg/L if done at 37 °C.[124]

Fosphenytoin is the IV prodrug of phenytoin; fosphenytoin pharmacokinetics and related monitoring are based on phenytoin calculations.[125-127]

Sample timing. The time required to attain a steady state after initiation of phenytoin therapy is difficult to predict due to phenytoin's nonlinear elimination behavior. Although the $T_{50\%}$ is approximately 24 hours (considering the average population V_{max} and K_m values when concentrations are between 10 and 20 mg/L), there can be extreme variations in these population values. Half-lives between 6 and 60 hours have been reported in adults.[94] Thus, a steady state might not be attained for as long as three weeks. Some clinicians advise that samples be obtained prior to steady state (after three to four days) to make sure that concentrations are not climbing too rapidly.[120] Equations have been developed to predict the time required to reach a steady state once V_{max} and K_m values are known.[50] It is important to recognize that the time required to reach a steady state in a given patient will be longer each time the dose rate is further increased.

Most clinicians advise that trough phenytoin concentrations be monitored.[94] Phenytoin is quite slowly absorbed so that the concentration versus time profile is fairly flat. This is especially true when oral phenytoin is administered two or three times per day. In this case, a serum phenytoin sample drawn any time during the dosage interval is likely to be close to a trough concentration. The greatest fluctuation would be seen for the more quickly absorbed products (chewable tablets and suspension) in children (who have a higher clearance of phenytoin) given once daily. In this case, it is particularly important to document the time of sample relative to the dose—to identify if the concentration is closer to a peak, a trough, or a $C_{ss,avg}$.

Use of concentrations for dosage adjustment. Phenytoin exhibits pronounced nonlinear behavior following therapeutic doses. Thus, increases in dose rate will produce greater-than-proportional increases in the average serum concentration during the dosing interval. Several methods, described elsewhere, use population and patient-specific V_{max} and K_m values to predict the most appropriate dose rate adjustment.[50] The clinician must be aware that the size of phenytoin daily dose increases should typically not be >30 or 60 mg using sodium phenytoin or 25 or 50 mg using the chewable tablets.

Protein binding, active metabolites, and other considerations. The metabolites of phenytoin have insignificant activity. Phenytoin binds primarily to albumin in plasma and the normal unbound fraction of drug in plasma of adults is 0.1.[94,120] Lower serum binding of phenytoin is observed in neonates and infants and in patients with hypoalbuminemia, liver disease, nephrotic syndrome or in end-stage renal disease (ESRD), pregnancy, cystic fibrosis, burns, trauma, malnourishment, AIDS, and advanced age.[84,128] Concurrent drugs (VPA, salicylate, and other nonsteroidal anti-inflammatory drugs [NSAIDs]) are known to displace phenytoin.[84] Thus, a total concentration of phenytoin that is within the range of 10–20 mg/L in these patients might represent an unbound concentration that is higher than 1–2 mg/L (the therapeutic range of unbound concentrations). A total concentration of phenytoin in this situation can be misleading. Several approaches can be used in these situations: (1) an unbound phenytoin concentration can be ordered, if available; (2) the patient's unbound phenytoin concentration can be calculated by estimating the unbound fraction in the patient (using the literature) and multiplying that by the patient's measured phenytoin concentration (the resulting unbound concentration should then be compared to 1–2 mg/L); or (3) special equations may be used to convert the PHT concentration to what it would be if the patient had normal serum protein binding.

The following equation was developed to normalize PHT concentrations in patients with hypoalbuminemia and renal failure[84,120,129]:

$$\begin{array}{l}\text{normalized}\\ \text{PHT concentration}\end{array} = \frac{\text{measured PHT concentration}}{(\text{X} \times \text{albumin concentration, g/dL}) + 0.1}$$

The value "X" is 0.2 for patients with low albumin and creatinine clearances equal to or above 25 mL/min, and 0.1 for patients with normal or low albumin who are receiving dialysis. Total concentrations of phenytoin in patients with creatinine clearance values between 10 and 25 mL/min cannot be as accurately normalized; the clinical status of such patients should be carefully considered because total concentrations can be misleading. This equation for normalizing PHT concentrations has been tested by groups of investigators in different groups of patients with mixed reviews; it is emphasized that it should be used only as a guide.

Known to increase the unbound fraction of phenytoin in serum, VPA also has been variably reported to inhibit the metabolism of phenytoin.[130] These two occurrences together could mean that a concentration within the range of 10–20 mg/L is associated with adverse effects and an unbound phenytoin concentration >2 mg/L. If unbound phenytoin concentrations are not available, the following equation—modified from its original form—may be useful to normalize the PHT concentration if the concentration of VPA in that same sample has been measured.[55,120]

$$\begin{aligned}&\text{normalized PHT concentration} = \text{measured PHT concentration}\\ &\quad + (0.01 \times \text{VPA concentration} \times \text{measured PHT concentration})\end{aligned}$$

Other equations have been used for estimating the unbound phenytoin concentration in the presence of VPA.[131] Salivary concentrations of phenytoin are strongly predictive of unbound phenytoin concentrations and have the added advantage of being noninvasive and thus an option for children and elderly patients.[58]

Valproic Acid

Therapeutic range. In addition to partial and generalized tonic-clonic and myoclonic seizures, VPA is used for management of absence seizures and for a variety of other conditions, including prophylaxis against migraine headaches and bipolar disorder.[94] Most laboratories use 50–100 mg/L as the therapeutic range for trough total VPA concentrations in the treatment of epilepsy; concentrations of 50–125 mg/L are considered therapeutic in the treatment of mania.[132] Some patients with epilepsy are effectively treated at lower concentrations, and others may require trough concentrations as high as 120 mg/L.[133] Concentrations at the upper end of the therapeutic range appear to be necessary for treatment of complex partial seizures.[133] The same therapeutic range has been used for patients with migraines or bipolar disorder, although the value of routine serum concentration monitoring for bipolar disorder has been questioned.[134] The following concentration-related side effects may be seen: ataxia, sedation, lethargy, and fatigue at concentrations above 75 mg/L; tremor at concentrations above 100 mg/L; and stupor and coma at concentrations >175 mg/L.[133] The therapeutic range of total VPA concentrations is confounded by the nonlinear serum protein binding of this drug, which might explain some of the variable response among and within patients at a given total serum concentration.[84]

Sample timing. The half-life of VPA ranges between 7 and 18 hours in children and adults and 17 and 40 hours in infants.[94] Thus, as long as five days may be required to attain a steady state. The pattern of change in VPA concentrations varies from interval to interval during the day because of considerable diurnal variation.[94,133] It is, therefore, recommended that samples always be obtained prior to the morning dose as the trough concentration has been shown to be most consistent from day to day.[133] Considerable fluctuation within the interval will be seen with the immediate-release capsule and syrup, which are rapidly absorbed. The enteric-coated, delayed-release Depakote tablet displays a shift-to-the-right with respect to its concentration versus time profile, such that the lowest concentration during the interval may not be observed until four to six hours into the next dosing interval.[102] It is important to know, however, that concentrations during the interval following administration of the enteric-coated tablet will show considerable fluctuation. The extended-release formulations (e.g., Depakote ER and Sprinkle capsules), if given in divided doses, provide less fluctuation in concentrations, and samples may be drawn at any time.

Use of concentrations for dosage adjustment. The metabolism of unbound VPA is linear following therapeutic doses. Thus, unbound VPA concentrations will increase in proportion to increases in dose rate.[84,102] Because VPA shows nonlinear, saturable protein binding in serum over the therapeutic range, total concentrations will increase less than proportionally. This is important to keep in mind when interpreting total VPA concentrations.

Protein binding, active metabolites, and other considerations. It is 90–95% bound to albumin and lipoproteins in serum. The unbound fraction of VPA shows considerable interpatient variability. It is increased in neonates, in conditions in adults associated with hypoalbuminemia (e.g., liver disease, nephrotic syndrome, cystic fibrosis, burns, trauma, malnutrition, and advanced age) and as a result of displacement by endogenous substances (e.g., bilirubin, free fatty acids, and uremic substances in ESRD) and other drugs (e.g., salicylate and other NSAIDs).[84,135,136] The increase in the unbound fraction of VPA during labor is believed to be the result of displacement by higher concentrations of free fatty acids.[137] It also shows intrapatient variability in the unbound fraction due to nonlinear binding. The unbound fraction of VPA is fairly constant at lower concentrations but progressively increases as total concentrations rise above 75 mg/L.[94] Thus, total concentrations do not reflect unbound concentrations at the upper end of the therapeutic range. A therapeutic range for unbound VPA concentrations only can be approximated. Assuming unbound fractions of 0.05–0.1 and a therapeutic range of total VPA concentrations of 50–100 mg/L, an unbound therapeutic range of 2.5–10 mg/L can be deduced.

Other Antiepileptic Drugs

Routine serum concentration monitoring is not recommended for most of the antiepileptic drugs.[138,139] The following drugs' serum concentrations would only be monitored if toxicity or nonadherence was suspected:

- **Ethosuximide.** The therapeutic range for ethosuximide is generally considered to be 40–100 mg/L.[140] Eighty percent of patients will achieve partial control within that range, and 60% will be seizure-free. Some patients will require concentrations up to 150 mg/L.[84] Side effects are usually seen at concentrations above 70 mg/L and include drowsiness, fatigue, ataxia, and lethargy.[84] Ethosuximide does not require as much monitoring as some of the other antiepileptics, but it is important to ensure effective concentrations after initiation of therapy or a change in dosage regimen. The half-life of ethosuximide is quite long—60 hours in adults and 30 hours in children.[102] Thus, it is advised to wait as long as one week to 12 days before obtaining ethosuximide concentrations for monitoring purposes.[15,140] Although it is generally advised that trough concentrations be obtained, concentrations drawn anytime during the dosing interval should be acceptable because there will be very little fluctuation if ethosuximide is given in divided doses. Peak concentrations of ethosuximide administered as a capsule are attained in three to seven hours.[102,140] Ethosuximide is negligibly bound to serum proteins and its metabolites have insignificant activity. Although ethosuximide is administered as a racemic mixture, the enantiomers have the same pharmacokinetic properties. Thus, measurement of the summed enantiomers is acceptable.[141]
- **Felbamate.** The therapeutic range of felbamate is considered to be between 30–60 mg/L. Dose decreases of up to 50% should be considered in patients with renal dysfunction.[142] Clinically, this medication is reserved to refractory treatment due to its side effect profile and increased incidence of aplastic anemia and acute liver failure.[142] Felbamate takes three to five days for reach steady-state concentration and serum concentrations should not be drawn before this time. Felbamate is 22–25% bound to albumin and has no active metabolites. Felbamate decreases the concentration of carbamazepine and increases the concentration of carbamazepine 10, 11-epoxide, phenobarbital, phenytoin, and VPA when administered with these agents; a 20–25% dose reduction should be considered when initiating felbamate with one of the aforementioned medications.[143-147] Simultaneously, coadministration of carbamazepine, phenobarbital, and phenytoin decreases felbamate concentrations, and VPA does not have a significant effect on felbamate concentrations.[142]
- **Gabapentin**. The therapeutic range of gabapentin is 2–10 mcg/mL; the toxic range is ≥25 mcg/mL. Dosing is based on creatinine clearance as this medication is renally eliminated.[148] Steady state is achieved within 24–48 hours.[149,150] Gabapentin is minimally bound to protein (<3%) and is eliminated 99% unchanged (1% is excreted as the N-methyl metabolite).[151] It is recommended that serum concentrations of concomitant antiepileptic therapies are monitored if given with gabapentin.[152]
- **Lamotrigine.** The considerable pharmacokinetic variability among patients taking lamotrigine, due in part to significant drug–drug interactions, makes it a good candidate for therapeutic drug monitoring.[138,153] The therapeutic range of lamotrigine is 2.5–15 mg/L.[27,139,154] It has been suggested that concomitant therapy with other antiepileptics may alter the response to lamotrigine or

its side effect profile.[153] The half-life of lamotrigine can range from 15–30 hours on monotherapy.[138] Thus, one should wait at least one week before obtaining samples after initiating or adjusting lamotrigine therapy.[153] This drug exhibits linear pharmacokinetics; therefore, dose rate adjustments will result in proportional changes in average serum concentrations. Because it is only 55% bound to serum proteins, measurements of unbound lamotrigine concentrations in serum are not necessary.

- **Levetiracetam**. Serum concentration monitoring of levetiracetam is more important in pregnancy and in infants and children because of the higher clearances in these patients.[27,155] The half-life ranges from six to eight hours in adults and a steady state should be attained within a week.[139] Serum concentrations between 5–45 mcg/mL are considered to be therapeutic.[156] Serum protein binding of levetiracetam is <10%, obviating the need for measurement of unbound levetiracetam concentrations.[139]
- **Oxcarbazepine**. The pharmacologic effect of oxcarbazepine is primarily related to serum concentrations of its active monohydroxy metabolite, licarbazepine. A therapeutic range of licarbazepine is 12–35 mg/L.[157] The elimination half-life of licarbazepine is quite variable, ranging from 7–20 hours, and is prolonged in renal impairment. The serum protein binding of licarbazepine is low at 40%.
- **Pentobarbital.** As a sedative hypnotic, therapeutic effects of pentobarbital are seen at 1–5 mg/L; toxicity occurs at concentrations >10 mg/L. Pentobarbital is 45–70% bound to protein and is primarily eliminated via the kidneys. Therapeutic concentrations have not been established for antiepileptic purposes.[158]
- **Pregabalin.** Pregabalin is approved as adjunctive treatment for partial onset seizures, and for the treatment of fibromyalgia; or diabetic, spinal cord injury-related, and postherpetic neuralgia. It is primarily eliminated via the kidneys and therefore requires dose adjustment in patients with kidney dysfunction. There are no known significant active metabolites.[159] Pregabalin reaches a steady state concentration at 24–48 hours.[149,150] The therapeutic range has been defined as 2–5 mcg/mL.[160]
- **Tiagabine**. Tiagabine shows pronounced interpatient pharmacokinetic variability. Trough concentrations between 20 and 100 mcg/L are associated with improved seizure control, but there is wide variation in response at any given total concentration.[27,138,139] This could, in part, be due to variable serum binding (96% bound in serum on average). Salicylate, naproxen, and VPA have been shown to displace tiagabine from serum proteins.[138,139] Tiagabine half-life ranges from 5–13 hours and may be even shorter in the presence of enzyme-inducing drugs.[139,155] Tiagabine shows linear elimination behavior following therapeutic doses.
- **Topiramate**. Topiramate concentrations are particularly influenced by interactions with other drugs, with concentrations as much as twofold lower when enzyme-inducing drugs are administered concurrently.[161] The half-life ranges from 18–23 hours, and it has linear elimination behavior.[138,139,153] Topiramate is <40% bound to serum proteins but shows saturable binding to red blood cells, thus suggesting that whole blood might be a preferable specimen for monitoring.[139,153] Effective serum concentrations are generally reported to be between 2–25 mcg/mL.[162] No active metabolites have been identified.
- **Zonisamide**. The pharmacokinetics of zonisamide are variable among patients and also highly influenced by interactions with other drugs.[138] Zonisamide is approximately 40% bound to serum albumin, and, like topiramate, shows saturable binding to red blood cells, suggesting that whole blood monitoring might be preferable.[139,153] The half-life is 50–70 hours but may be as short as 25 hours when enzyme inducers are coadministered.[138] There are some reports suggesting nonlinear behavior at higher doses. The serum concentration range associated with response is 10–38 mg/L; cognitive dysfunction is reported at concentrations above 30 mg/L.[27,139,153] No active metabolites have been identified.[138]

Antimicrobials

Aminoglycosides

Therapeutic ranges. Aminoglycosides have been used for decades to treat infections caused by multidrug- resistant microorganisms. Most commonly utilized IV aminoglycosides today include amikacin, gentamicin, and tobramycin.[49] They are bactericidal and their efficacy is highly dependent on peak concentration following an infusion.[163] Optimal bactericidal activity occurs when the peak:MIC ratio is between 8:1 to 10:1.[164-166] They also exhibit a postantibiotic effect (PAE) in which bacterial killing continues even after the serum concentration is below the minimum inhibitory concentration (MIC).[84] The concentration-dependent killing and PAE of aminoglycosides explain why extended-interval, or once-daily (pulse) dosing of aminoglycosides is shown to be safe and effective in many patients. Studies have shown improved peak concentrations of aminoglycosides following high-dose, once-daily dosing of aminoglycosides when compared to multiple daily dosing regimens (i.e., thrice-daily dosing). Additionally, lower trough concentrations have been reported at the end of the dosing regimen compared to multiple daily dosing regimens.[167] As a result, extended-interval aminoglycosides, including gentamicin, have been associated with improvement in efficacy and decreased nephrotoxicity.[168-171] Nephrotoxicity and ototoxicity are the most frequently reported adverse effects of the aminoglycoside antibiotics. Ototoxicity seems to be associated with a prolonged course of treatment (for >7–10 days) with peaks above 12–14 mg/L for gentamicin and tobramycin and 35–40 mg/L for amikacin.[84] One study noted that there was not a significant difference in the incidence of ototoxicity between once-daily and multiple daily dosing aminoglycosides.[167] Patients with trough concentrations above 2–3 mg/L (gentamicin, and tobramycin) or 10 mg/L (amikacin) for sustained periods of time (greater than five to seven days) are predisposed to increased risk of nephrotoxicity.[84] The risk of nephrotoxicity is even further increased when aminoglycosides are given concomitantly with other nephrotoxic agents.

Therapeutic ranges for peaks and troughs are reported for the aminoglycosides and pertain only to dosing approaches that involve multiple doses during the day. For gentamicin and tobramycin, peaks between 6 and 10 mg/L and troughs between 0.5 and 2 mg/L are recommended.[172] The approximately fourfold higher MIC for amikacin explains why peaks between 20 and 30 mg/L and troughs between 1 and 8 mg/L are recommended.[172] There is no therapeutic range when the pulse-dosing method is used; doses are given to attain peaks that are approximately 10 times the MIC, and troughs are intended to be nondetectable within four hours of administration of the next dose.[163,172] A serum concentration drawn sometime after infusion of the dose is used only for adjustment of the dosing interval, not to check for efficacy or toxicity.

There has been some concern over the years that aminoglycosides are over-monitored. Uncomplicated patients who have normal renal function, do not have life-threatening infections, and will be treated for less than five days may not need to have serum aminoglycoside concentrations measured.[173] At the other extreme, dosage individualization using serum concentrations of aminoglycosides is necessary in patients who are expected to be on prolonged treatment courses (greater than or equal to five days), or in whom unusual pharmacokinetic parameters are expected.[172-177]

Sample timing. For extended-interval dosing patients with normal renal function, a steady state is never reached because each dose is washed out prior to the next dose. The method developed by Nicolau et al. (the Hartford nomogram) requires that a single blood sample be obtained between 6 and 14 hours after the end of the first infusion.[164,178] This sample is referred to as a *random sample*, but the time of the collection must be documented. The concentration is used with a nomogram to determine if a different dosing interval should be used.[49,164] Concentrations that are too high according to the Hartford nomogram will indicate that the drug is not being cleared as well as originally predicted, suggesting the need for a longer dosing interval. For traditional dosing, it is important to wait until a steady state is reached before obtaining blood samples. The half-lives of the aminoglycosides are one and a half to three hours for adults with normal renal function but as long as 72 hours in patients with severe renal impairment.[84] Because the dosing interval for aminoglycosides is usually adjusted to be two to three times the drug's half-life, then a conservative rule of thumb is that steady state is reached after the third or fourth dose.[49] Some patients may have blood samples drawn immediately after the first dose ("off the load") to determine their pharmacokinetic parameters for purposes of dosage regimen individualization. These would most likely be patients who are anticipated to have unpredictable or changing pharmacokinetic parameters, such as those in a critical care unit, and who require immediate effective treatment because of life-threatening infections.

Two blood samples are sufficient for purposes of individualizing traditional aminoglycoside therapy, and will provide reasonable estimates of aminoglycoside pharmacokinetic parameters.[181] It is crucial that the times of the sample collections be accurately recorded.[49,163] The two samples should be spaced sufficiently apart from each other so that an accurate determination of the log-linear slope can be made to determine the elimination rate constant. The first sample, sometimes referred to as the *measured peak,* should be drawn no earlier than one hour after the end of a 30-minute infusion (at minimum).[179] A second sample may be drawn any time later.[180,181] However, it is usually drawn within 30 minutes of the start of infusion of the next dose (assumed to be the trough).[49,164,173] Once the elimination rate constant has been calculated using these two concentrations, the true peak and true trough can be calculated and their values compared to desired target peaks and troughs.

Use of concentrations for dosage adjustment. Various extended-interval dosing methods are used to take advantage of the concentration-related killing and PAE of aminoglycosides.[173] The original Hartford method involves giving a mg/kg dose that is administered to attain a peak concentration that is approximately 10 times the MIC. Then a sample is obtained between 6 and 14 hours after the end of the infusion and compared to a nomogram, which indicates the appropriate maintenance dosing interval—usually 24, 36, or 48 hours.[164] Extended-interval dosing methods are not routinely recommended for certain patients, including those with enterococcal endocarditis (i.e., aminoglycoside synergy dosing), renal failure, meningitis, osteomyelitis, or burns.[173] However, studies are ongoing to show safety and efficacy in more subpopulations of patients. The results of clinical trials do not consistently show a reduction in nephrotoxicity, and it has been proposed that extended-interval doses be lowered to provide daily AUCs similar to those measured following traditional daily doses.[163,169]

Serum concentrations of aminoglycosides obtained during traditional dosing are used to determine an individual patient's pharmacokinetic parameters, as well as the true peak and true trough to compare these to desired target concentrations. Equations that account for time of drug infusion are used to determine an appropriate dosing interval and dose.[182] Other dosage adjustment methods include nomograms and population pharmacokinetic (Bayesian) methods.[174,182]

Protein binding, active metabolites, and other considerations. Aminoglycosides are <10% bound to serum proteins, and unbound concentrations will always reflect total concentrations in serum.[172] The metabolites of the aminoglycosides are inactive.

Vancomycin

Therapeutic range and clinical considerations. Vancomycin is a glycopeptide antibiotic that is used intravenously to treat gram-positive organisms, including those resistant to other antibiotics (i.e., methicillin-resistant *Staphylococcus aureus* [MRSA]).[49,84] Emergence of vancomycin-resistant enterococci, vancomycin-resistant *Staphylococcus aureus*, vancomycin-intermediate *Staphylococcus aureus* (VISA), and heteroresistant VISA. *S. aureus* has led to the need to optimize and restrict its use. The major toxicities associated with vancomycin are nephrotoxicity and ototoxicity (likely aggravated by concurrent administration of other nephrotoxic and ototoxic agents). Another adverse effect known as *red man syndrome* (intense flushing, tachycardia, and hypotension) is usually associated with rapid infusion.[84,183]

Although some institutions may monitor both peaks and troughs of vancomycin, this practice has been questioned because of a lack of standardization in when a sample is drawn to appropriately reflect a "peak." In contrast to aminoglycosides, it is more important to have sustained vancomycin concentrations above the MIC during the dosing interval than it is to have high peaks and low troughs. Higher clinical success rates have been noted in patients in whom the ratio of the 24 hour AUC to MIC is approximately 400. However, it is almost impossible to achieve this pharmacodynamic parameter in isolates with MICs of 2 or greater with conventional vancomycin dosing.[184] It is recommended that trough concentrations for vancomycin be kept above 10 mg/L to avoid the development of resistance.[185,186] Higher trough concentrations of 15–20 mg/L are recommended for complicated infections including bacteremia, endocarditis, osteomyelitis, meningitis, and pneumonia caused by *S. aureus*, including MRSA.[187,188] Although it is sometimes recommended that peak concentrations must be kept below 50 mg/L to avoid ototoxicity, this recommendation is based on only two cases.[163] It is more likely that ototoxicity is due to excessively high total vancomycin exposure.

Vancomycin is routinely monitored in all patients in some hospitals, but many question the need for this in uncomplicated infections (i.e., cellulitis) and in noncritically ill patients with normal renal function.[49,163] Conflicting evidence exists related to the relationship of serum concentrations, efficacy outcomes, and safety (nephrotoxicity and ototoxicity) outcomes.[189-203] Indications for monitoring include patients with unstable renal function (changing renal function or on renal replacement therapy), patients at high risk for nephrotoxicity (i.e., if receiving other nephrotoxic agents concurrently); patients expected to have unusual pharmacokinetics (e.g., burns, malignancies, and IV drug abusers); patients on prolonged courses of therapy (i.e., greater than five days); and patients with suboptimal response.[49,204-207]

Sample timing. The half-life of vancomycin is seven to nine hours in adults with normal renal function, but it can be as long as 120–140 hours in patients with renal failure. Vancomycin's half-life is approximately 7 hours in full-term neonates, 6 hours in children, and 12 hours in patients older than 65.[207] Half-life is three to four hours in obese patients and four hours in burn patients.[84] Samples should be obtained as troughs, within 30 minutes to one hour of the start of the next infusion. Samples should be taken once the medication is at steady state with q 12 hr dosing in a patient with normal renal function; this translates to drawing a trough prior to the fourth dose.[208]

Use of concentrations for dosage adjustment. Vancomycin elimination is linear, and an increase in the dose (without a change in the dosing interval) can be expected to provide a proportional change in the trough serum concentration. It must be cautioned that vancomycin has a very pronounced distribution phase, making the standardization of any peak sample to be especially important. More sophisticated prediction methods for dosing adjustments must be used if the dosing interval is adjusted with or without a change in dose.

Many methods have been proposed for vancomycin dosage regimen adjustments.[84,89,209,210] A relatively simple method proposed by Ambrose and Winter permits the use of a single trough concentration (drawn within one hour of the start of the next infusion) along with an assumption of the population distribution volume to predict the necessary pharmacokinetic parameters needed for individualization.[183] Once those parameters are determined, equations that account for drug infusion can be used to target desired peak and trough vancomycin concentrations.

Protein binding, active metabolites, and other considerations. Vancomycin is 30–55% bound to serum proteins in adults with normal renal function. The binding is lower (19%) in patients with ESRD.[210] With binding this low, total concentrations of vancomycin provide reliable indications of the unbound concentrations in serum. Vancomycin metabolites are inactive, and, thus, do not contribute to antibacterial effect or toxicity.

β-Lactams

Therapeutic range and clinical considerations. Early administration of appropriate antibiotics has great implications on outcomes and survival in patients presenting with sepsis and septic shock. Often therapy includes broad spectrum antibiotics with β-lactams being the most commonly prescribed in this scenario.[211] The β-lactam antibiotics demonstrate time-dependent bactericidal killing with efficacy related to the percentage of the dosing interval that free drug concentration remains above the MIC (fT>MIC). Studies have shown that the killing potential of β-lactams is maximized at concentrations that are three to four times the MIC with higher concentrations providing little benefit.[212] Therefore, it is important to optimize exposure of β-lactams by increasing dosing frequency or infusion duration. Generally, fT>MIC of 40%, 50%, and 50–70% are required for carbapenems, penicillins, and cephalosporins bactericidal killing potential, respectively.[213] As demonstrated by an increase in V_d and changes in antibiotic clearance (either increased or decreased), β-lactam antibiotic pharmacokinetics may be altered in critically ill patients. Additionally, obesity can similarly alter antibiotic pharmacokinetics parameters. As a result, standard doses of antibiotics may be suboptimal, leading to inadequate concentrations and even toxicities in some situations.[214] Roberts and colleagues noted that one fifth of critically ill patients did not achieve a PK/PD (pharmacokinetic/pharmacodynamic) target of 50% fT>MIC when standard antibiotic doses were used.[211] Historically, therapeutic drug monitoring of β-lactams was not undertaken because these agents lack a narrow therapeutic window and toxicity that would necessitate monitoring. In fact, an estimate of only ~30 hospitals worldwide currently perform routine β-lactam therapeutic drug monitoring with wide variability in how this is conducted. However, as the incidence of multidrug-resistant microorganisms continues to increase, dosing optimization of currently available antibiotics is more important than ever and provides a potential role for β-lactam therapeutic drug monitoring, especially in the critically ill patient population and other patients with known pharmacokinetic variability.[212]

Antifungal Agents

Flucytosine (5-FC)

Therapeutic range and clinical considerations. Flucytosine is a synthetic antifungal agent that is used in combination with amphotericin B for treatment of select systemic fungal infections (i.e., cryptococcal meningitis).[215] It is also used increasingly in combination with the azole antifungal agents and is part of therapeutic approaches used for the treatment of certain tumors, such as colorectal carcinoma.[216] In vivo and animal studies have noted that time above MIC is the most important pharmacodynamic parameter related to outcome with flucytosine therapy.[217] Most clinicians agree that peak serum concentrations (two-hour postdose) of flucytosine should be kept below 100 mg/L to avoid dose-related hepatotoxicity, bone marrow suppression, and gastrointestinal disturbances.[163,216,218] Some clinicians also advise that trough concentrations of flucytosine be kept between 25 and 50 mg/L (or kept above 25 mg/L) to avoid rapid development of resistance.[163,216,218] If a constant infusion is used, steady-state serum concentrations of 50 mg/L should be targeted.[163] The hepatotoxicity and bone marrow suppression are both usually reversible with discontinuation. Indications for monitoring flucytosine include avoidance of toxicity—particularly in patients with impaired renal function or those receiving concomitant amphotericin B—and avoidance of resistance due to sustained low concentrations.[163,215-217]

Sample timing. Flucytosine is minimally protein bound (2–4%) and undergoes minimal hepatic metabolism. It is primarily (90%) excreted in the urine as unchanged drug, with an elimination half-life of four to five hours in patients with normal renal function and upwards of 250 hours in ESRD.[216,217,219] A two-hour postdose concentration of flucytosine should be obtained after three to five doses have been administered, noting that steady state may not be reached for approximately 10 days in patients with renal failure.[215,217] Trough concentrations, if indicated, should be drawn within 30 minutes of the next dose.

Use of concentrations for dosage adjustment. Because there are no reports of nonlinear elimination behavior, a given increase in dose rate or infusion rate should produce a proportional increase in serum flucytosine concentration.

Azole Antifungals

Therapeutic ranges and clinical considerations. The incidence of fungal infections has been on the rise as the number of patients at risk has increased (e.g., patients receiving immunosuppressive therapy). Azole antifungal agents are used to treat a number of different fungal infections including invasive candidiasis, aspergillosis, and mucormycosis. The primary reason for monitoring azole antifungal drugs is to ensure efficacy and safety as they have demonstrated wide interpatient pharmacokinetic variability. Suboptimal azole antifungal concentrations have been associated with treatment failure and fungal breakthrough, while high concentrations have been related to toxicities (e.g., hepatotoxicity).

Itraconazole. Itraconazole concentrations are known to be relatively low in patients with AIDS or acute leukemia, most likely due to malabsorption and concurrent administration of enzyme-inducing drugs.[220] Additionally, absorption of the oral capsule formulation is greatly dependent on the gastric pH; itraconazole demonstrates improved absorption in an acidic environment. This necessitates the administration of the oral capsule with a full meal or acidic drink (e.g., cola). Conversely, the oral liquid solution's absorption is improved when not taken with food.[217] Itraconazole displays nonlinear pharmacokinetics. It accumulates slowly and reaches concentrations of 0.5–1 mg/L after one to two weeks. Additionally, itraconazole inhibits CYP3A4, leading to a number of significant drug interactions.[221] Due to interpatient and absorption variability based on formulation used, some consider the serum concentration monitoring of itraconazole to be essential in patients with life-threatening fungal infections.[222] It has been noted that mortality and breakthrough infections are more common with itraconazole trough concentrations <0.5 mg/L, and toxicity with concentrations >3 mg/L. Efficacy has been associated with itraconazole concentrations of 0.5–1 mg/L as measured by HPLC methods. It is recommended to measure itraconazole concentrations to ensure adequate absorption, monitor for need for dosage change (e.g., when interacting medications are added or discontinued), and to assess adherence to therapy. Due to a long elimination half-life (~34–42 hours after multiple doses), the concentration of itraconazole can be drawn at any time during a dosing interval once steady state is reached (approximately two weeks).[223,224]

Voriconazole. Voriconazole is a first-line treatment option for invasive aspergillosis and other invasive fungal Infections. It exhibits nonlinear pharmacokinetics related to saturable clearance mechanisms. This leads to greatly variable and unpredictable changes in drug exposure secondary to dosage adjustments.[217] Voriconazole has excellent bioavailability, however it is metabolized by a number of significant CYP450 enzymes (i.e., CYP2C9, CYP3A4, and CYP2C19). These enzymes may have significant interpatient variability due to enzyme polymorphism and therefore lead to varying voriconazole concentrations.[217] Suboptimal voriconazole concentrations have been associated with suboptimal response and treatment failure. Additionally, elevated voriconazole concentrations have been correlated with toxicities which include hepatotoxicity, visual disturbances, and hallucinations. Based on available data, voriconazole concentrations between 1 mg/L and 4–6 mg/L are recommended to increase efficacy and decrease risk of toxicity. A trough concentration (e.g., end of 12-hour dosing interval) should be drawn within the first week of therapy initiation or dosage adjustment.[217,221,225]

Posaconazole. Posaconazole is indicated for the treatment of a number of invasive fungal infections, including mucormycosis.

Posaconazole is available as an oral suspension, delayed-release tablet, and IV solution.[226] The oral formulations (oral suspension and delayed-release tablet) are not interchangeable because of noted pharmacokinetic differences. The oral formulations are influenced by food intake. Additionally, the oral suspension is influenced by gastric pH. It is recommended that the oral suspension be given with a high-fat meal to enhance bioavailability (by 2.6–4 times).[221,225] Similar to previously mentioned azole antifungals, posaconazole demonstrates large interpatient variability in its pharmacokinetic parameters. An exposure-toxicity relationship is still unknown for posaconazole.[217,225] Due to the prolonged half-life of posaconazole (~26–35 hours), steady state is not reached until the end of first week of therapy, and serum concentrations can be measured at any time during the interval at that point.[221,225] Greater clinical response to posaconazole has been related to higher drug concentration exposure. Although a recommended trough concentration has not been defined, some have suggested a trough of >0.7 or >0.9 mcg/mL for prophylaxis, and a trough of >0.7 or ≥1.8 mcg/mL for treatment. These apply to the delayed-release or IV formulation.[221]

Fluconazole. Therapeutic drug monitoring of fluconazole is not required due to predictable concentrations based on currently available data. Additionally, it is less affected by drug–drug interactions. Of note, an AUC/MIC ratio >25 is related to improved clinical outcomes. [217,222]

Antimycobacterials

The optimal use of therapeutic drug monitoring for mycobacterial infections is currently under study. Drugs that are FDA-approved and considered first line as part of an initial four-drug regimen are isoniazid, rifampin, pyrazinamide, and either ethambutol or streptomycin. Of these, isoniazid and rifampin are the most important based on their relatively high potency and favorable side-effect profiles. Second-line agents that are more toxic must be used if drug resistance emerges and include ethionamide, cycloserine, capreomycin, para-aminosalicylic acid, and dapsone.[227]

Therapeutic drug monitoring is not routine in the treatment of active tuberculosis (TB). However, studies have noted commonly observed low concentrations of isoniazid and rifampin, which can lead to slow response, relapse, and drug resistance.[228] As a result, it is essential that adequate concentrations of these antimycobacterial drugs be present in serum for effective treatment and avoidance of negative consequences. This does not always occur, even in patients in whom adherence has been documented.[227] Lower-than-expected concentrations of antimycobacterial drugs have been reported in patients with diabetes and in those with HIV infections, which in some cases was associated with malabsorption.[229-231] There is also considerable potential for drug–drug interactions among the antimycobacterial drugs, given the effects of rifampin, isoniazid, and the fluoroquinolones in either inducing or inhibiting cytochrome P450 isozymes.[232] Drugs used to treat HIV patients may also contribute to this drug–drug interaction quagmire.

A study in non-HIV infected TB patients who were not responding to treatment as expected showed that 29–68% of them had serum antimycobacterial drug concentrations below target ranges.[233] In another study, a small percentage of non-responding patients all showed suboptimal concentrations of rifampin.[234] After dosage adjustments were made, all patients responded to treatment. The authors recommended that low serum rifampin concentrations be suspected in patients who do not respond after three months of supervised drug administration or earlier in patients with HIV infection, malnutrition, known gastrointestinal or malabsorptive disease, or hepatic or renal disease.

Most TB drugs display AUC/MIC as the most important pharmacodynamic parameter; however, the relationship between dose or serum concentrations and toxicity are not well established (exceptions include pyrazinamide, ethambutol, and cycloserine). A number of TB drugs display significant interpatient variability necessitating therapeutic drug monitoring of these agents to avoid potential associated treatment failure, relapse, and toxicity.[235]

Specialized laboratories have been developed that offer sensitive and specific assays for serum concentrations for the most commonly used antimycobacterial drugs.[227] As more specific information about the efficacy of therapeutic drug monitoring of these drugs becomes available, more laboratories and services of this type will likely be available.[236]

Antiretrovirals

Therapeutic ranges and clinical considerations. Overall, there is a lack of published literature supporting improved clinical outcomes in HIV-infected adults with therapeutic drug monitoring. Additionally, significant interpatient variability exists in regard to pharmacokinetics. Therapeutic concentrations, therefore, have not been established for most antiretrovirals.[237,238] Although routine monitoring of antiretroviral agents is not recommended, there are some scenarios in which therapeutic drug monitoring should be considered. These include instances where significant drug–drug or drug–food interactions may lead to reduced efficacy or toxicities; physiological and anatomical changes (e.g., gastrointestinal) that may impair drug absorption or metabolism, pregnant women not achieving virologic clearance, and heavily treatment-experienced patients who have developed virologic failure.[239] There is some evidence that favors limited serum concentration monitoring of drugs used in the treatment of HIV-1 infection, in particular the PIs and the nonnucleoside reverse-transcriptase inhibitors (NNRTIs).[240,241] These drugs, particularly the PIs, show marked interpatient variability in their pharmacokinetics and their serum concentration concentrations correlate with virologic response and failure.[242,243] A substudy of the randomized, prospective clinical trial, AIDS Therapy Evaluation in the Netherlands (ATHENA), showed that patients who underwent serum drug concentration monitoring for the antiretroviral drugs had a significantly higher likelihood of virological response as compared to those who did not undergo monitoring.[242]

Minimum concentration (C_{min}) is the proposed target concentration parameter.[239] Minimum effective concentrations have been determined for the most common PIs based on in vitro determinations of drug concentrations (corrected for serum binding) required for 50% or 90% inhibition of replication in the patient's virus isolate (IC_{50} or IC_{90}). Attention has turned more recently, however, to the use of a new parameter that may be a better predictor of response. The inhibitory quotient (IQ) is the ratio of the patient's trough plasma concentration to the IC_{50} or IC_{90}.[242] A high IQ indicates more drug is present in the patient than is needed for virologic response, while a low IQ indicates inadequate drug concentrations or a resistant virus. Recent studies show virologic response may be better related to IQ than to trough concentrations alone.[242] Future studies may focus on the definition of therapeutic ranges of IQ rather than minimum concentrations.

The most commonly used PIs are fosamprenavir (a prodrug of amprenavir), darunavir, atazanavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir and tipranavir. The most commonly used NNRTIs are efavirenz, nevirapine and etravirine. Serum concentrations of newer agents (enfuvirtide, a fusion inhibitor, and raltegravir, an integrase inhibitor) may also be monitored in select situations.[240,241] Some clinicians advocate the monitoring of these drugs in all patients on initiation of therapy to ensure adequate concentrations; others reserve use for selected situations including patients with renal or liver disease, pregnancy, children, patients at risk for drug interactions, and suspected toxicity.[242,244]

Sample timing. Half-lives of the NNRTIs average 25–50 hours, and steady state will be reached after a week in most patients.[245] However, a steady state will be reached within two days for the PIs, which have half-lives ranging from 2–12 hours.[245] Predose samples are recommended as the minimum effective concentrations and the IQs are based on the lowest drug concentration during the dosing interval. There may be logistical problems with this timing, however, in cases when the drug is administered once daily in the evening. Some drugs, such as nelfinavir, exhibit a lag in their absorption, such that the lowest concentration actually occurs about an hour after administration of the next dose.

Use of concentrations for dosage adjustment. Dosage adjustments of the antiretroviral drugs, for the most part, should result in proportional changes in the trough serum drug concentration, provided the dosing interval is not altered. Reports showing serum drug concentrations to be unpredictable after dosage adjustments in some patients suggest that nonadherence with antiretroviral regimens is a major concern.[242] Serum concentrations of amprenavir, lopinavir, nelfinavir, and saquinavir may be difficult to maintain above their minimum effective concentrations because of rapid clearances and large first-pass effects. Rather than increasing their dose rate, ritonavir, a potent inhibitor of CYP3A4-mediated metabolism in the gut wall and liver, may be coadministered as a pharmacoenhancer. This results in decreased gastrointestinal enzyme metabolism of the PI, higher trough concentrations, and, in most cases, prolonged elimination half-lives.[246]

Protein binding, active metabolites, and other considerations. The serum protein binding of nevirapine and indinavir is 50% to 60%, while the protein binding of the other antiretrovirals is >90%.[242,245] Albumin and AAG are the primary binding proteins for these drugs in serum.[242] As would be expected, there is considerable variability in the unbound fraction of these drugs in serum. In addition, AAG concentrations are elevated in patients with HIV-1 infection and can return to normal with treatment. Thus, the same total concentration of the drug would be expected to reflect a lower concentration of response early in treatment as compared to later. Clearly, total concentrations of the PIs and NNRTIs should be cautiously interpreted if unusual serum binding is anticipated, but no clear guidelines are yet available. Only nelfinavir has a metabolite that is known to be active.[242] Although studies indicate the measurement of the metabolite is probably not crucial, there is likely to be considerable variability among and within patients in the presence of this metabolite.

Cardiac Drugs

Digoxin

Therapeutic range and clinical considerations. There has been a dramatic reduction in digoxin toxicity since the advent of therapeutic drug monitoring for digoxin.[247] Routine monitoring is not necessary unless digoxin toxicity is expected, the patient has declining renal function, a suspected change in pharmacokinetics due to changing condition, or initiation of concomitant interacting medications.[248] Additionally, patients with electrolyte abnormalities (hypokalemia, hypomagnesemia, and hypercalcemia), hypothyroidism, myocardial ischemia, and acidotic states are at higher risk of toxicity. Patients with hyperthyroidism are believed to be more resistant to digoxin.[30]

Digoxin's inotropic effect is the basis for its use for treatment of congestive heart failure, while its chronotropic effects are the basis for treatment of atrial arrhythmias such as atrial fibrillation, atrial flutter, and paroxysmal atrial tachycardia. The commonly reported therapeutic range is 0.5–2 mcg/L in adults and 1–2.6 mcg/L in neonates.[87,247,249] The lower end of the range (0.5–0.9 mcg/L) is generally used for treatment of heart failure.[250] Results of the Digitalis Investigation Group (DIG) trial found that concentrations of 0.5–0.8 mcg/L in men with heart failure (ejection fraction <46%) reduced hospitalizations in a posthoc analysis.[251] Dosing strategies for patients with heart failure have been established based on kidney function, ideal body weight, and height.[252-254] Higher serum digoxin concentrations are required for treatment of atrial arrhythmias (0.8–1.5 mcg/L), with additional benefit gained in some patients with concentrations up to 2 mcg/L.[84]

Fifty percent of patients with serum digoxin concentrations above 2 mcg/L show some form of digoxin toxicity; toxicity may be experienced at lower concentrations and management should be based on symptoms.[84,255] Symptoms of toxicity include muscle weakness; gastrointestinal complaints (anorexia, nausea, vomiting, abdominal pain, and constipation); CNS effects (headache, insomnia, confusion, vertigo, and changes in color vision); and serious cardiovascular effects (second-degree or third-degree atrioventricular [AV] bradycardia, premature ventricular contractions, and ventricular tachycardia) (**Table 6-4**).[84,249]

TABLE 6-4. Digoxin Toxicities[256]

CARDIAC EFFECTS	• Arrhythmias • Sinus bradycardia
CNS/GI EFFECTS	• Anorexia, nausea, vomiting, abdominal pain • Visual disturbances: halos, photophobia, color perception dysfunction (red-green or yellow green), scotomata • Fatigue, weakness, dizziness, headache, confusion, delirium, psychosis

CNS = central nervous system; GI = gastrointestinal.

Sample timing. The average digoxin half-life in adults with normal renal function is approximately two days; at least seven days are recommended to attain a steady state.[249] In the case of treatment of digoxin overdose with digoxin-immune Fab fragments (a fragment of an antibody that is very specific for digoxin), blood samples for serum digoxin measurements should not be obtained sooner than 10 days after administration of the fragments.[247,249]

Samples drawn during the absorption and distribution phases after administration of digoxin cannot be appropriately interpreted by comparison to the usual therapeutic range. Digoxin concentrations in blood do not reflect the more important concentrations in myocardial tissue until at least six hours after the dose (some say at least 12 hours).[249,257,258] Blood samples should, therefore, be drawn anytime between 6 hours after the dose and right before the next dose (**Figure 6-5**).

Inappropriate timing of samples for digoxin determinations is problematic. One study showed that 55% of the samples submitted to the laboratory for digoxin analysis lacked clinical value because of inappropriate timing.[259] In another study, standardization of digoxin administration and blood sampling times resulted in a dramatic reduction in inappropriately timed samples (i.e., timed at 1700 for digoxin administration and timed at 0700 for blood sampling.[260] Another recommendation is that the laboratory immediately contact the clinician if digoxin concentrations are above 3.5 mcg/L.[249] If it is confirmed that the sample was drawn too early after the dose, another sample should be requested. If monitoring is considered at initiation of therapy, serum concentrations should be drawn within 12–24 hours of loading dose; if no loading dose is administered, clinicians should wait three to five days after therapy initiation to evaluate concentrations.[261]

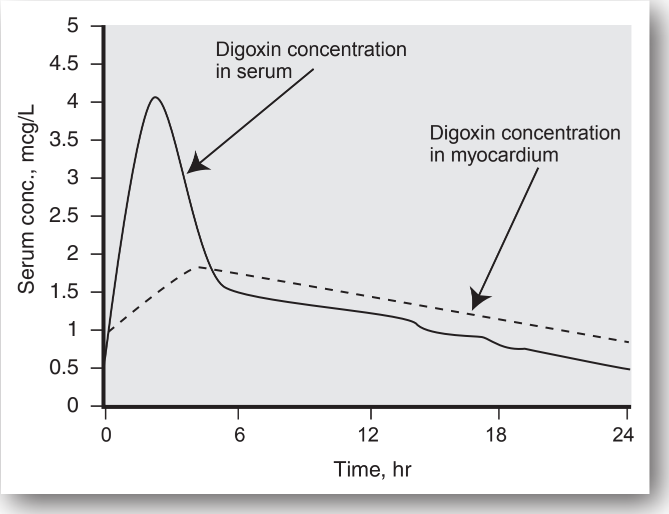

FIGURE 6-5. Simulated plot showing concentrations of digoxin in serum (mcg/L) and concentrations in myocardial tissue (units not provided) after a dose of digoxin at steady state. Tissue concentrations do not parallel concentrations in serum until at least six hours after the dose.

Use of concentrations for dosage adjustment. Dose rate adjustments of digoxin based on serum digoxin concentrations are straightforward. Because of linear elimination behavior, a given increase in the daily digoxin dose will produce a proportional increase in the serum concentration at that time during the dosing interval. To determine steady state concentrations after dose adjustments, obtain serum concentration 5–7 days after any dose change, and 7–14 days thereafter until therapeutic concentrations are reached. In patients with ESRD, steady state may not be obtained for 15–20 days.[261]

Protein binding, active metabolites, and other considerations. Digoxin is only 20–30% bound to serum proteins.[30] Therefore, total concentrations in serum will reflect the pharmacologically active unbound concentration. The biologic activity of digoxin metabolites is modest compared to the parent drug, and variable presence of metabolites should not affect the interpretation of a digoxin serum concentration.

Other Cardiac Drugs

Amiodarone. Amiodarone is used for the treatment of life-threatening recurrent ventricular arrhythmias that do not respond to adequate doses of other antiarrhythmics. The primary metabolite, desethylamiodarone (DEA), has similar electrophysiologic properties as amiodarone and accumulates at concentrations similar to or higher than the parent drug, especially in renal failure patients.[247] Concentrations of amiodarone and DEA demonstrate linear pharmacokinetics with increasing doses of amiodarone.[262] The concentration versus effect relationship for amiodarone is poorly defined; some say that serum concentrations between 0.5 and 2.5 mg/L are associated with effectiveness with minimal toxicity.[247] The occurrence of toxicity has been reported at plasma concentrations >2.5 mg/L.[263-265] Laboratories that measure serum amiodarone concentrations report only the parent drug, despite high concentrations of the active metabolite. In general, therapeutic drug monitoring of amiodarone is of limited benefit because activity of the drug is mostly associated with concentrations in the tissue.[247] Serum concentrations might be most useful in cases of suspected nonadherence or toxicity.

Lidocaine. Lidocaine is a type 1B antiarrhythmic used as second-line therapy for the acute treatment for ventricular tachycardia and fibrillation. The therapeutic range is generally considered to be 1.5–5 mg/L with concentrations >6 mg/L considered to be toxic.[84,249,266] Minor side effects—drowsiness, dizziness, euphoria, and paresthesias—may be observed at serum concentrations above 3 mg/L. More serious side effects observed at concentrations above 6 mg/L include muscle twitching, confusion, agitation, and psychoses, while cardiovascular depression, AV block, hypotension, seizures, and coma may be observed at concentrations above 8 mg/L.[84,249,267] Lidocaine is not monitored as commonly with short term use because its effect (abolishment of the electrocardiogram [ECG]-monitored arrhythmia) is easy to directly observe. Indications should be restricted to situations in which the expected response is not evident or when decreased hepatic clearance is suspected or anticipated: liver disease, congestive heart failure, advanced age, severe trauma, and concurrent drugs such as β-adrenergic blockers, fluvoxamine, or cimetidine.[247,249,266] Additionally, serum concentrations should be monitored in the case of extended use, although infusion duration beyond six hours is not recommended.[268] The half-life of lidocaine ranges from one and a half hours to as long as five hours in patients with liver disease.[84,267] Thus, steady state may not be attained for 18–24 hours even if a loading dose is administered. Because lidocaine is administered as a continuous infusion, there are no fluctuations in concentrations, and blood lidocaine serum concentrations can be drawn anytime once steady state is reached. Adjustments of lidocaine infusion rate should result in a proportional increase in lidocaine serum concentration. The unbound percentage of lidocaine is normally 30% but can range from 10% to 40% due to variations in AAG concentrations.[84,249] The combination of higher total concentrations of lidocaine during prolonged infusions and a lower unbound fraction mean that unbound lidocaine concentrations during prolonged infusions are probably therapeutic.[269] It is important to be aware that total lidocaine concentrations at the higher end of the therapeutic range may not present a danger of toxicity in patients receiving prolonged infusions of lidocaine after a myocardial infarction. The monoethylglycinexylidide metabolite of lidocaine has 80–90% of the antiarrhythmic potency of lidocaine, and its concentration accumulates in renal failure.[84,266]

Procainamide. Although the oral formulation is no longer available in the United States, the IV form of procainamide is used for patients with atrial fibrillation or flutter who require cardioversion.[270,271] The therapeutic range of procainamide is complicated by the presence of an active metabolite, NAPA, which has different electrophysiologic properties than the parent drug. Procainamide is a type 1A antiarrhythmic, while NAPA is a type III antiarrhythmic.[84,247,249] The enzyme that acetylates procainamide is bimodally distributed, such that patients are either slow or fast acetylators. In addition, NAPA is more dependent on the kidneys for elimination than is procainamide.[249,272] Most patients respond when serum procainamide concentrations are between 4 and 8 mg/L; some receive additional benefit with concentrations up to 12 mg/L.[270] There have been reports of patients requiring concentrations between 15 and 20 mg/L without adverse effects.[270] Serum concentrations of NAPA associated with efficacy are reported to be as low as 5 mg/L and as high as 30 mg/L. Most clinicians consider toxic NAPA concentrations to be above 30–40 mg/L.[249] Some clinicians feel that NAPA does not need to be monitored except in patients with renal impairment.[270] Most laboratories automatically measure both procainamide and NAPA concentrations in the same sample. The practice of summing the two concentrations and comparing it to a therapeutic range for summed procainamide and NAPA (often reported as 10–30 mg/L) is to be discouraged.[84,249,270] To do this validly, the molar units of the two chemicals would need to be used.[250] The best practice is to independently compare each chemical to its own reference range.[84,247,249] Side effects to procainamide and NAPA are similar. Anorexia, nausea, vomiting, diarrhea, weakness, and hypotension may be seen with procainamide concentrations above 8 mg/L, while concentrations above 12 mg/L may be associated with more serious adverse effects: heart block, ventricular conduction disturbances, new ventricular arrhythmias, and even cardiac arrest.[84] Indications for procainamide and NAPA serum concentration monitoring include recurrence of arrhythmias that were previously controlled, suspected toxicity or overdose, anticipated pharmacokinetic alterations caused by drug–drug interactions (including amiodarone, cimetidine, ethanol, ofloxacin, quinidine, ranitidine, and trimethoprim), and disease state changes (renal failure or congestive heart failure, in particular).[84,249,270,272] The half-life of procainamide in adults without renal impairment or congestive heart failure ranges from two and a half hours (fast acetylator) to five hours (slow acetylator).[84,249] The half-life of NAPA is longer, averaging six hours in patients with normal renal function and 30 hours or longer in patients with renal impairment.[84,270] Thus, a steady state of both chemicals is not observed until at least 18 hours in patients with good renal function or as long as four days in renal impairment. The lower clearance of procainamide at higher dose rates has been attributed to nonlinear hepatic clearance.[273] The clinician should be aware that increases in infusion rate may produce somewhat greater-than-proportional increases in serum procainamide concentration in some patients, particularly those with serum concentrations at the upper end of the therapeutic range. Procainamide is only 10–20% bound to serum proteins.[247,250] Thus, total procainamide and NAPA concentrations always reflect the pharmacologically active unbound concentrations of these drugs.

Quinidine. The therapeutic range of quinidine for treatment of severe malaria due to *Plasmodium falciparum* is reported as 3–6 mg/L.[274] When used in combination with verapamil for prevention of atrial fibrillation, the therapeutic range of quinidine is reported to be 2–6 mg/L, although use of quinidine is not strongly recommended due to a threefold increased risk of cardiac death when compared to other antiarrhythmic agents.[275-277] Common side effects are anorexia, nausea, and diarrhea; more serious side effects include cinchonism, hypotension, and ventricular arrhythmias.[84,249] Torsades de pointes is more likely to occur at lower concentrations of the therapeutic range, thus complicating the interpretation of quinidine concentrations.[247] Indications for monitoring of quinidine concentrations include therapeutic confirmation; suspected toxicity; recurrence of arrhythmias; drug–drug interactions; suspected nonadherence; and changes in formulation.[84,249,277] The half-life of quinidine is reported to range from four to eight hours in adults and up to 10 hours in patients with liver disease. Steady state should be attained within two or three days in patients with normal hepatic function, and most clinicians agree that samples should be drawn as a trough within one hour of the next dose.[84,247,249,277] Quinidine is a weak base that is normally between 70–80% bound to albumin and AAG.[249] The unbound fraction of quinidine was shown to be decreased in patients with atrial fibrillation or atrial flutter, and the unbound quinidine concentration was shown to correlate better with ECG interval changes than total quinidine.[278,279] A total quinidine concentration that is above 5 mg/L could be therapeutic with respect to unbound quinidine concentration. The dihydroquinidine impurity may be present in amounts that are between 10–15% of the labeled amount of quinidine and is believed to have similar electrophysiologic properties as quinidine.[249] The 3-hydroxyquinidine metabolite has activity that is less than the parent (anywhere between 20–80% have been reported), is less highly bound to serum proteins, and demonstrates accumulation with chronic treatment.[272,280]

Cytotoxic Drugs

Although cytotoxic drugs have some characteristics that make them ideal candidates for therapeutic drug monitoring (narrow therapeutic indices and variable pharmacokinetics) they have many more characteristics that make therapeutic drug monitoring difficult or unsuitable.[281,282] They lack a simple, immediate indication of pharmacologic effect to aid definition of a therapeutic range (the ultimate outcome of cure could be years). They are given in combination with other cytotoxic drugs, such that concentration versus effect relationships for any single drug is difficult to isolate. They are used to treat cancer, which is a highly heterogeneous group of diseases, each possibly having its own concentration versus effect relationships. In summary, cytotoxic drugs are not routinely monitored because they are in need of more clearly defined therapeutic ranges. If ranges are established, they are usually more helpful to avoid toxicity than to define zones for efficacy.

Methotrexate

Therapeutic range. Methotrexate is the only antimetabolite drug for which serum concentrations are routinely monitored.[281] It acts by blocking the conversion of intracellular folate to reduced folate cofactors necessary for cell replication. Although cancer cells are more susceptible to the toxic effects of methotrexate, healthy host cells are also affected by prolonged exposure to methotrexate. It is for this reason that leucovorin, a folate analogue that prevents further cell damage, is administered following high-dose methotrexate treatments.[281] Measurements of serum methotrexate concentrations at critical times following high-dose methotrexate regimens are imperative to guide the amount and duration of leucovorin rescue treatments, thus preventing methotrexate toxicity. Institution of protocols for methotrexate serum concentration monitoring for this purpose has resulted in dramatic reductions in high-dose methotrexate-related toxicity and mortality.[283]

Although it is known that methotrexate concentrations must be sufficiently high to prevent relapse of the malignancy, the specific range of concentrations related to efficacy has been difficult to define.[283] However, the relationship between methotrexate concentrations and toxicity has been much more clearly defined. Prolonged concentrations of methotrexate can lead to nephrotoxicity, myelosuppression, gastrointestinal mucositis, and liver cirrhosis.[281,283] Serum concentration monitoring is not generally indicated when relatively low doses of methotrexate are given for chronic diseases such as rheumatoid arthritis, asthma, psoriasis, and maintenance for certain cancers.

Sample timing. The timing of samples for determination of methotrexate concentrations is highly dependent on the administration schedule. As one example of such a protocol, a methotrexate dose may be administered by IV infusion over 36 hours followed by a regimen of leucovorin doses administered over the next 72 hours.[284] Additional or larger leucovorin doses might be given depending on the methotrexate concentrations in samples drawn at various times after the start of the methotrexate infusion. It is important that methotrexate concentrations continue to be monitored until they are below the critical concentrations (usually between 0.05 μM and 0.1 μM).[281,284]

Use of concentrations for dosage adjustment. Methotrexate and leucovorin doses are based on protocols.

Protein binding, active metabolites, and other considerations. Methotrexate binds to albumin in serum ranges from 20% to 57%.[281] Although studies have shown the unbound fraction of methotrexate to be increased by concomitant administration of NSAIDs, salicylate, sulfonamides, and probenecid, the implications for interpretation of methotrexate concentrations are probably not important.[284] The methotrexate metabolite, 7-hydroxymethotrexate, has only 1/100th the activity of methotrexate but may cause nephrotoxicity due to precipitation in the renal tubules.[283]

Other Cytotoxic Drugs

Petros and Evans provide an excellent summary of cytotoxic drugs and the types of measurements that have been used to predict their toxicity and response.[283] Correlations between response or toxicity and serum concentration or AUC versus time curve for total drug have been shown for busulfan, carboplatin, cisplatin, cyclophosphamide, docetaxel, etoposide, 5-fluorouracil, irinotecan, paclitaxel, teniposide, topotecan, and vincristine.[283,285,286] The strong correlation between busulfan AUC and bone marrow transplant outcome led the FDA to include instructions for AUC monitoring in the package insert for IV busulfan. Unbound AUC values for etoposide and teniposide, which demonstrate concentration-dependent binding, correlate more strongly with toxicity than corresponding total plasma AUC values.[287] Systemic drug clearance has been predictive of response/toxicity for amsacrine, fluorouracil, methotrexate, and teniposide.[283,288] Steady-state average serum concentrations or concentrations at designated post-dose times have also been predictive of response/toxicity for cisplatin, etoposide, and methotrexate.[283] Finally, concentrations of cytosine-arabinoside metabolite in leukemic blasts and concentrations of mercaptopurine metabolite in red blood cells have been predictive of response or toxicity for these drugs. Correlations between systemic exposure and response/toxicity for cyclophosphamide, carmustine, and thiotepa have also been reported.[289] Toxicity of doxorubicin has been found to be associated with peak plasma concentrations.[290] Although most studies up to this point have focused on use of cytotoxic drug concentration measurements to minimize toxicity, future studies will be increasingly focused on use of drug concentrations to maximize efficacy.

Immunosuppressant Drugs

Cyclosporine

Therapeutic range and clinical considerations. Cyclosporine is a potent cyclic polypeptide used for prevention of organ rejection in patients who have received kidney, liver, bone marrow or heart transplants.[291] It is also used for the management of psoriasis, rheumatoid arthritis, and other autoimmune diseases. The therapeutic range of cyclosporine is highly dependent on the specimen (whole blood or serum/plasma) and assay. Most transplant centers use whole blood with one of the more specific assays—high-performance liquid chromatography (HPLC) or immunoassays that use monoclonal antibodies (monoclonal radioimmunoassay or monoclonal fluorescence polarization immunoassay).[84,292,293] The commonly cited therapeutic range for whole blood troughs using one of these specific methods is 100–500 mcg/L.[84,294] Troughs at the higher end of this range may be desired initially after transplantation and in patients at high risk for rejection.[294] The therapeutic range also depends on the specific organ transplantation procedure and the stage of treatment after surgery (higher concentrations during induction and lower concentrations during maintenance to minimize side effects).[84,293-296] Thus, it is important that the therapeutic range guidelines established by each center be used. Although most centers still use single trough concentrations to adjust cyclosporine doses, the area under the blood concentration versus time curve is believed to be a more sensitive predictor of clinical outcome.[297] Studies that have investigated the use of single cyclosporine concentrations measured two hours after the dose, as a surrogate for the AUC value, suggest a better clinical outcome as compared to the use of single trough concentrations.[292,297,298]

Cyclosporine has a narrow therapeutic index and extremely variable pharmacokinetics among and within patients. The implications of ineffective therapy and adverse reactions are serious. Thus, it is imperative that cyclosporine concentrations be monitored in all patients starting immediately after transplant surgery. The primary side effects associated with high cyclosporine blood concentrations are nephrotoxicity, neurotoxicity, hypertension, hyperlipidemia, hirsutism, and gingival hyperplasia.[84,294,296] Blood cyclosporine concentrations should also be monitored when there is a dosage adjustment, signs of rejection or adverse reactions, suspected nonadherence, or initiation or discontinuation of drugs known to induce or inhibit cyclosporine metabolism.[294,296]

Sample timing. Monitoring is often done immediately after surgery before a steady state is reached. Initially, concentrations may be obtained daily or every other day, then every three to five days, then monthly. Changes in dose rate or initiation or discontinuation of potential enzyme inducers or inhibitors will require resampling once a new steady state is reached. The half-life of cyclosporine ranges from 5–27 hours and is dependent on the particular formulation. Thus, every three to five days is generally adequate in most patients for attainment of a new steady state. Most centers continue to sample predose (trough) cyclosporine concentrations, while some are using two-hour postdose concentrations, which appear to more closely predict total exposure to cyclosporine as measured by AUC.[292,298] Multiple samples to determine the AUC are generally unnecessary.

Use of concentrations for dosage adjustment. In most cases, simple proportionality may be used for dosage adjustments. Trough or two-hour postdose concentrations will change in proportion to the change in dose rate so long as the dosing interval remains the same.

Protein binding, active metabolites, and other considerations. Cyclosporine is 90% bound to albumin and lipoproteins in blood.[295] Unbound fractions in blood vary widely among patients and are weakly correlated to lipoprotein concentrations in blood.[295] For example, lower unbound fractions of cyclosporine have been reported in patients with hypercholesterolemia.[296] Lindholm and Henricsson reported a significant drop in the unbound fraction of cyclosporine in plasma immediately prior to acute rejection episodes.[299] An association between low cholesterol concentrations (and presumably high unbound fractions of cyclosporine) and increased incidence of neurotoxicity has also been reported.[296] These studies suggest that efforts to maintain all patients within a certain range of total concentrations may be misleading. Routine monitoring of unbound cyclosporine concentrations is not yet feasible, given the many technical difficulties with this measurement. Instead, the clinician should cautiously interpret total concentrations of cyclosporine in situations where altered protein binding of cyclosporine has been reported.

Other Immunosuppressant Drugs

Everolimus. Everolimus is an mTOR (mammalian target of rapamycin) kinase inhibitor which is used in the treatment of many oncologic conditions, in patients with liver and renal transplants, and in a subependymal giant cell astrocytoma (SEGA).[300] Everolimus is primarily metabolized through CYP3A4 and has six known weak metabolites. The half-life is ~30 hours and bioavailability decreases with high-fat meals.[300,301] Whole blood concentrations should be in the range of 3–8 mcg/L for liver and renal transplant patients; 5–15 mcg/L is the reference range in patients with SEGA. In patients with liver or renal transplant, trough concentrations should be drawn four to five days after dose adjustments or after initiation, dose modification, or discontinuation of interacting medications; the everolimus dose should be adjusted at this time. In patients with SEGA, concentrations should be monitored approximately two weeks after initiation or dose adjustment, when interacting medications are initiated, discontinued, or changed, with changes in everolimus formulation, or with changes in liver function. When a maintenance dose has been attained, trough concentrations should be monitored every 6–12 months. More frequent monitoring of three to six months is suggested if the patient's body surface area is fluctuating. Everolimus is 74% protein-bound.[300]

Mycophenolic acid. Mycophenolate mofetil, the prodrug of mycophenolic acid, is often used in combination with cyclosporine or tacrolimus with or without corticosteroids.[296] Although troughs of mycophenolic acid may be monitored (plasma concentrations between 2.5 and 4 mg/L are targeted with good success), AUC values appear to be better predictors of postoperative efficacy (avoidance of acute rejection).[298,302,303] Reliable measurements of AUC may be determined with as few as three samples (trough, 30 minutes, and 120 minutes post-dose) with a desired target AUC range of 30–60 mg × hr/L.[302] The half-life of mycophenolic acid is approximately 17 hours. Thus, a new steady state will be attained approximately three days after a dose rate change or the addition/discontinuation of drugs that affect the metabolism of mycophenolic acid. Mycophenolic acid is 98% bound to plasma proteins, and the unbound fraction is greatly influenced by changes in albumin concentration, displacement by metabolites, renal failure, and hyperbilirubinemia.[292,302] Several groups of investigators suggest that unbound mycophenolic acid concentrations should be monitored when altered binding is suspected.[296,302-305] There is evidence that unbound mycophenolic acid concentration may be a better predictor of adverse effects than total concentration.[304,306]

Sirolimus. Sirolimus is a macrolide antibiotic with potent immunosuppressant activity. When used in combination with cyclosporine and corticosteroids, trough blood concentrations of 5–15 mcg/L are generally targeted.[294] With whole blood sampling, therapeutic concentrations measure 10–15 mcg/L with concomitant calcineurin inhibitors, and higher ranges of 15–25 mcg/L are targeted without calcineurin inhibitors.[307] It has a relatively long half-life (62 hours), and a new steady state will not be attained in many patients until at least six days after dose rate adjustments or the addition or discontinuation of interacting drugs.[294] At present, trough concentrations are used for monitoring as they correlate well with AUC.[292]

Tacrolimus. Tacrolimus is a macrolide antibiotic with immunosuppressant activity and is generally used in combination with other immunosuppressant drugs. Trough blood concentrations of tacrolimus as high as 20 mcg/L are targeted during initial treatment and gradually decrease to between 5 and 10 mcg/L during maintenance therapy, often after 12 months.[298] Toxicities to tacrolimus are very similar to those with cyclosporine, including nephrotoxicity and neurotoxicity.[294,308] The unpredictable and variable extent of tacrolimus bioavailability (5–67%) contribute to the need for monitoring of this drug.[308] Differences in pharmacogenomics (related to the CYP3A4*22 allele) have been associated with altered pharmacokinetics.[309] Monitoring should always be done after changes in dose rate or initiation/discontinuation of enzyme-inducing or inhibiting agents. The half-life of tacrolimus ranges from 4–41 hours, and a new steady state will be attained after approximately three to five days.[294,296] Although trough concentrations are still the method of choice for monitoring, a second concentration might be needed if Bayesian approaches to dosage individualization are used.[298,310] Tacrolimus appears to exhibit linear elimination behavior. Thus, an increase in the daily dose is expected to result in a proportional increase in the steady-state trough concentration. Tacrolimus is 75–99% bound to plasma proteins (albumin, α-1-glycoprotein, lipoproteins and globulins).[296,308] Reports of lower unbound serum concentrations of tacrolimus during episodes of rejection lead one to be cautious with interpretation of total tacrolimus concentrations in patients with suspected alterations in protein binding.[306]

Psychotropics

Amitriptyline, Nortriptyline, Imipramine, Desipramine

Therapeutic ranges. Although tricyclic antidepressants (TCAs) continue to be used for treatment of depression, their use has significantly declined in favor of the newer antidepressants.[311] The therapeutic ranges of amitriptyline, imipramine, desipramine, and nortriptyline are well defined.[26,312,313] Desipramine and nortriptyline are also active metabolites of imipramine and amitriptyline, respectively.

When imipramine is administered, combined serum concentrations of imipramine and desipramine that are considered therapeutic but not toxic are between 180 and 350 mcg/L.[312,313] Combined concentrations above 500 mcg/L are extremely toxic.[313] When desipramine is administered, concentrations between 115 and 250 mcg/L are frequently associated with therapeutic effect.[312,313] When amitriptyline is administered, combined serum concentrations of amitriptyline and nortriptyline should be between 120 and 250 mcg/L.[312] Combined concentrations above 450 mcg/L are not likely to produce additional response and are associated with cardiotoxicity and anticholinergic delirium.[313] The therapeutic range of nortriptyline is the most firmly established of these four drugs; target serum concentrations after nortriptyline administration are between 50 and 150 mcg/L.[312-314]

The most common side effects of the TCAs are anticholinergic in nature.[312] Toxicities seen at higher concentrations include cardiac conduction disturbances , seizures, and coma.[312] For all of the TCAs, these toxic effects occur at serum concentrations that are approximately five times those needed for antidepressant efficacy.[12] Indications for monitoring include suspected nonadherence or inadequate response, suspected toxicity, and suspected unusual or altered pharmacokinetics (children, elderly, and drug interactions).

The TCAs in general are highly bound to serum proteins.[311] Thus, one would expect unbound TCA serum concentrations to be much better predictors of response than total concentrations, particularly in populations suspected to have unusually high or low serum binding. Until now, studies that have attempted to examine this have not been able to clarify relationships between response and total serum concentrations based on variable protein binding. The TCAs are extensively metabolized and undergo significant first-pass metabolism. Although the primary active metabolites have been identified and are separately measured, other active metabolites can accumulate in some circumstances and affect the response at a given parent drug concentration.[315]

Lithium

Therapeutic range. Lithium is a monovalent cation used for the treatment of bipolar disorder, the manic phase of affective disorders, and is somewhat effective in the treatment of refractory unipolar depression.[316] The concentration units for lithium are expressed as mEq/L, which is the same as mmol/L. Although the overall therapeutic range for treatment of manic depression is commonly cited as 0.5–1.2 mEq/L, there appear to be two distinct ranges used in practice, depending on the stage of therapy.[26,312] For acute management of manic depressive episodes, the therapeutic range of 0.8–1.2 mEq/L is desired, going up to 1.5 mEq/L if necessary.[84,317,318] For maintenance treatment, the therapeutic range of 0.6–1 mEq/L or 1–1.2 mEq/L is usually recommended.[84,317,319,320] In elderly patients, target therapeutic concentrations are as low as 0.2 mEq/L.[321] Serum concentrations above 1.5 mEq/L are associated with fine tremors of the extremities, gastrointestinal disturbances, muscle weakness, fatigue, polyuria, and polydipsia. Concentrations above 2.5 mEq/L are associated with coarse tremors, confusion, delirium, slurred speech, and vomiting. Concentrations above 2.5–3.5 mEq/L are associated with seizures, coma, and death.[312] It is important to point out that the values for the therapeutic ranges are based on samples obtained at a specific time during the day—just before the morning dose and at least 12 hours after the evening dose for patients on a BID or TID regimen.[317,322]

Most clinicians require that every patient taking lithium be regularly monitored, which is cost-effective considering the potential avoidance of toxicity.[84,314] Specific indications for lithium concentration monitoring include evaluation of nonadherence; suspicion of toxicity; confirmation of the concentration associated with efficacy; and any situation in which altered pharmacokinetics of the drug is anticipated (drug–drug interactions, pregnancy, children, geriatric patients, and fluid and electrolyte imbalance). Despite the strong indication for lithium monitoring in all patients, 37% of lithium users on Medicaid did not have serum drug concentrations monitored.[323]

Sample timing. The half-life of lithium ranges from 18–24 hours, and steady state will be reached within a week of therapy.[317] However, two to three weeks of treatment may be required after that before the full response to the drug can be assessed.[317] When initiating lithium therapy, it is recommended that serum concentrations be measured every two to three days (before a steady state is reached) to ensure that concentrations do not exceed 1.2 mEq/L during that time.[84] Because of the extreme variability of serum lithium concentrations during the absorption and distribution periods, the current standard of practice is to draw all samples for lithium serum concentration determination 12 hours after the evening dose, regardless of whether a twice or thrice daily dosing schedule is used. For example, the time for blood sampling for a patient on a 0900/1500/2100 schedule would be right before the 0900 dose.[84] The timing of blood samples for a patient taking once daily lithium is less clear, given the greater degree of serum lithium concentration fluctuation with this dosing method. [317]

Use of concentrations for dosage adjustment. Lithium exhibits linear elimination behavior and proportionality can be assumed when dosage adjustments are made. The assumption of linearity is the basis for several dosing methods that are used for initiating lithium therapy in patients. The Cooper method involves drawing a sample for lithium analysis 24 hours after a first dose of 600 mg.[45] The resulting concentration, believed to provide a reflection of the drug's half-life, is used with a nomogram that indicates the optimal maintenance regimen. The Perry method requires that two concentrations be drawn during the postabsorption, postdistribution phase after a first dose of lithium.[46] These two concentrations are used to determine the first-order elimination rate constant, which can then be used to determine the expected extent of lithium accumulation in the patient. The maintenance regimen required to attain a desired target lithium concentration in that patient can then be determined. Population-pharmacokinetic, dosing-initiation methods (Bayesian) can also be used.[84]

Protein binding, active metabolites, and other considerations. Lithium is not bound to serum proteins, nor is it metabolized.

Other Psychotropics

Antidepressants. Assays have been developed to document the serum concentrations observed following administration of other cyclic antidepressants as well as the selective serotonin reuptake inhibitors, serotonin and norepinephrine reuptake inhibitors, and norepinephrine reuptake inhibitors.[12,313,319,324-326] Although reference ranges have been established, there does not appear to be any compelling reason for routine monitoring of these drugs given their relatively wide therapeutic indices and more favorable side effect profiles. Because 50% of patients do not achieve optimal relief from symptoms of depression, some clinicians advocate the use of serum concentration monitoring in patients who do not initially respond to identify nonadherence or to identify unusually low serum concentrations.[12,26,315,316]

Antipsychotics. The existence of well-defined therapeutic ranges for most antipsychotic drugs remains controversial.[327-329] There is some justification, however, for serum concentration monitoring of clozapine, fluphenazine, haloperidol, olanzapine, perphenazine, risperidone, and thioridazine in special circumstances.[26,330,331,332] Clozapine demonstrates clinical response for most patients at concentrations >350 mcg/L, but increasing serum concentrations above this range are associated with symptoms of toxicity.[333] Although there is a range of interpatient variability related to clozapine pharmacokinetics, Rajkumar and colleagues found that in patients with treatment-resistant schizophrenia, increasing doses of clozapine, caffeine intake, and VPA administration were most closely associated with serum clozapine concentrations.[331] Additionally, haloperidol has a therapeutic range of 5–20 mcg/L for the treatment of psychotic disorders and is considered toxic at concentrations >42 mcg/L. Lower serum concentrations are targeted in Tourette syndrome and mania.[332] Reference ranges for other antipsychotic drugs are primarily based on average serum concentrations observed during chronic therapy.[314,319]

One difficulty in establishing clear therapeutic range guidelines is that chronicity of illness and duration of antipsychotic drug exposure can shift the therapeutic range; separate therapeutic ranges may need to be developed depending on duration of illness.

FUTURE OF TDM

Drug assays are rapidly improving with regard to specificity, sensitivity, speed, and convenience. Methods that separate drug enantiomers may help to elucidate therapeutic ranges for compounds administered as racemic mixtures.[334] Capillary electrophoresis-based assays will be increasingly used in clinical laboratories because of their low cost, specificity, utility for small sample volumes, and speed.[335] Methods for measurement of drugs in hair samples are being proposed for assessment of long-term drug adherence.[336] Implanted amperometric biosensors, currently used for glucose monitoring, may be useful for continuous monitoring of drug concentrations.[337] Subcutaneous microdialysis probes may also be useful for continuous drug monitoring, particularly because they monitor pharmacologically active unbound drug concentrations.[338] Point-of-care assay methods, currently used in private physician offices, group practices, clinics, and EDs, could eventually be used in community pharmacies in the future.[339,340]

The therapeutic drug monitoring of the near future may also involve determination of genotypes, characterization of proteins produced in particular diseases (proteomics), and analysis of drug metabolite profiles (metabonomics).[341,342] These sciences may help to identify those subsets of patients who will be nonresponders or toxic responders and help to determine appropriate initial doses. Such testing would not require special sample timing, might be possible using noninvasive methods (e.g., hair, saliva, and buccal swabs), and would need to be done only once as the results would apply over a lifetime. These types of testing may help patients receive the best drug for the indication and rapid individualization of drug dosage to achieve desired target concentrations.[33,34,341,343] This will likely result in increased demand for new types of tests from clinical laboratories currently involved in routine therapeutic drug monitoring.

There is a movement to change the terminology and practice of therapeutic drug monitoring to *target concentration strategy, target concentration intervention,* or *therapeutic drug management*.[47,344] Critics of the therapeutic drug monitoring terminology claim that it suggests a passive process that is concerned only with after-the-fact monitoring to ensure that concentrations are within an ill-defined range without proper regard to evaluation of the response to the drug in an individual patient.[344] Target concentration intervention is essentially a new name for a process used by clinical pharmacokinetics services for years and involves the following steps: (1) choose a target concentration (usually within the commonly accepted therapeutic range) for a patient; (2) initiate therapy to attain that target concentration using best-guess population pharmacokinetic parameters; (3) fully evaluate response at the resulting steady-state concentration; and (4) adjust the regimen as needed using pharmacokinetic parameters that have been further refined by use of the drug concentration measurement(s).

The desire for positive clinical responses with new biologic agents such as monoclonal antibodies warrants further investigation as patients seek individualized and expensive treatment options. Studies do not currently recommend routine monitoring, but clinicians should be aware of further development in this area.[345,346] Methods to improve the therapeutic drug monitoring process itself are needed. Every effort should be made to focus on patients who are most likely to benefit from therapeutic drug monitoring, and minimize time and money spent on monitoring that provides no value.[9] The biggest problems with the process continue to be lack of education, communication, and documentation.[6,24] Approaches to changing physician behavior with regard to appropriate sampling and interpretation include educational sessions, formation of formal therapeutic drug monitoring services, multidisciplinary quality improvement efforts, and computerization of requests for drug concentration measurement samples.[347] Pharmacists will continue to have a pivotal role in the education of physicians and others involved in the therapeutic drug monitoring process. Future studies that evaluate the effect of therapeutic drug monitoring on patient outcomes will likely use quality management approaches.[348]

LEARNING POINTS

1. ***A female patient who was diagnosed with complex partial seizures was initiated on VPA, 750 mg/day. She returns to the clinic after four weeks and reports that she has not had any seizures since taking the VPA. There are no signs or symptoms consistent with VPA toxicity. Why should a serum VPA concentration be measured in this patient?***

   ANSWER: Because the endpoint of therapy is the absence of something (seizures in this case), there is no way to ensure that the patient is taking enough VPA. Some types of seizures occur infrequently, and it is possible that the patient's serum VPA concentration is low and she simply hasn't had a seizure yet. It is important to ensure that the concentration is within the therapeutic range of 50–100 mg/L (for patients with normal serum albumin concentrations) before assuming the patient is adequately protected from future seizure activity

2. ***A 24-year-old female patient with hypoalbuminemia has been initiated on phenytoin for treatment of generalized tonic-clonic seizures. A serum phenytoin concentration is measured and reported as 16 mg/L. The patient reports that she has not experienced any seizures since starting the phenytoin, but she's noticed weakness and blurry vision since then. Nystagmus is observed on physical exam. The laboratory reports a serum albumin concentration of 2.5 g/dL (normal is 3.5–5 g/dL). How do you interpret the phenytoin concentration?***

ANSWER: The target therapeutic range for PHT concentrations is reported as 10–20 mg/L. This range, however, assumes an albumin concentration that is within the normal range. A patient with abnormally low albumin concentrations is likely to show toxicity when PHT concentrations are between 10 and 20 mg/L. This is because the unbound concentration of phenytoin is too high. It is likely in this case that the dose of phenytoin is too high, thus accounting for the ataxia and nystagmus. A PHT concentration at the low end of the usual therapeutic range (or even below) would be a more appropriate goal.

3. ***A 60-year-old male patient with normal renal function was initiated on oral digoxin, 0.25 mg every morning, for treatment of supraventricular arrhythmias. He returned to his primary care physician one month later for an 8 a.m. appointment. A blood sample, drawn at 8:30 a.m., revealed a digoxin serum concentration of 2.9 mcg/L. There are no signs or symptoms of digoxin toxicity. On further inquiry, the patient reveals that he took his digoxin dose at 7:30 a.m. that morning. A repeat sample drawn right before the next digoxin dose is 1.2 mcg/L.***

   ANSWER: This illustrates the importance of blood sample timing relative to intake of the last drug dose. The serum digoxin concentration in this case is above the upper limit generally defined for patients with atrial arrhythmias (2 mcg/L), and might lead to the conclusion that the daily digoxin dose is excessively high. However, the sample should have been drawn sometime between six hours after the dose and right before the next dose, when the digoxin in blood will have equilibrated with digoxin in myocardial tissue. Significant resources are wasted by inappropriate timing of blood samples for digoxin measurements, and incomplete documentation of dose or blood sample timing.

4. ***A 45-year-old male patient is postoperative day 2 following a living-donor kidney transplant. He was initiated on IV tacrolimus yesterday. Labs were drawn this morning and revealed a concentration of 25 mcg/L. Due to the unexpected supratherapeutic concentration, the clinical pharmacy specialist requests the concentration to be redrawn. A repeat concentration is 4 mcg/L.***

   ANSWER: Appropriateness of drawn concentrations is key in interpreting them. There are a number of reasons why a concentration may not make sense. Dosed appropriately, it is expected that the tacrolimus concentration in this patient will be on the lower end of the therapeutic range because a steady state is unlikely to have been attained one day after initiation of therapy. One possible explanation for the supratherapeutic concentration of tacrolimus is an improper blood draw procedure. It's possible that the blood was drawn from an administration line that was not flushed prior to sample withdrawal. Misinterpretation of concentrations due to inappropriate draws can have dire consequences if dosing changes are made based on falsely elevated or low concentrations.

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7

PHARMACOGENOMICS AND MOLECULAR TESTING

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OBJECTIVES

After completing this chapter, the reader should be able to

- Define pharmacogenetics
- Differentiate germline and somatic mutations
- Understand the use of molecular testing in pharmacogenetics/genomics as tools for personalizing therapy
- Describe the difference between empirical pharmacotherapy and genotype-enhanced pharmacotherapy
- Understand how pharmacogenetics can enhance therapeutic drug monitoring
- Assess the utility of genotype in addition to other patient-specific factors for specific medications in the provision of pharmaceutical care
- Discuss the role of laboratory medicine in pharmacogenetics in terms of turnaround time, interpretative reporting, and assay performance

PHARMACOGENETICS

As early as the 1950s, the heritable nature of drug response was noted for agents such as succinylcholine, isoniazid, and primaquine.[1-3] Later, twin studies confirmed this heritability by showing that the half-lives of some drugs were tightly correlated in monozygotic twins and had little correlation in dizygotic twins.[4] Since that time, the fields of pharmacogenetics and pharmacogenomics have taken off, and the genetic basis for variability in drug metabolism, transport, and pharmacodynamic effect is increasingly being appreciated. In fact, pharmacogenetic and molecular tests are routinely used in therapeutic areas such as hematology/oncology, and their usefulness is being explored in every major therapeutic drug class.[5]

Pharmacogenetics/pharmacogenomics is the translational science of correlating interindividual genetic variation with variability in drug response. Historically and practically, the terms *pharmacogenetics* and *pharmacogenomics* have been used interchangeably (as in this chapter). However, definitions may vary depending on the context. For example, pharmacogenetics can be seen as the study of variants in a handful of candidate genes. Contrarily, because of our expanding technological ability to simultaneously investigate millions of variants across the human genome using genome-wide genotyping arrays or high-throughput sequencing, pharmacogenomics may refer to genome-wide investigation of drug response variability.

Pharmacogenetics seeks to avoid adverse drug reactions and improve clinical efficacy, providing personalized medicine to patients, much the same way therapeutic drug monitoring by serum drug concentrations customizes certain medication regimens for individual patients. One goal of pharmacogenetics is to refine the current empirical approach to drug therapy management so that it is less "trial-and-error" in nature. There are often many drug classes available to treat a given condition, and several drugs within each of those classes that a clinician may opt to use. This large armamentarium of drug therapy choices can lead to an inefficient, time-consuming management strategy in which the therapeutic decision is based on little more than clinician preference. Another goal of pharmacogenetics is to provide the appropriate dose to individual patients so that the "one dose fits all" strategy is avoided. Incorporating the results of genetic tests along with nongenetic factors (e.g., age, sex, smoking status, interacting drugs, and others) into pharmacotherapy decision making may help streamline this process such that the likelihood for response is maximized while the chance of toxicity is minimized.[6]

Understanding the results of molecular tests that are used in the application of pharmacogenetics is of critical importance to healthcare providers if this form of personalized medicine is going to improve patient care. Many institutions are attempting to implement preemptive genotyping so that results will be in the electronic medical record before a particular drug with a useful genetic test is prescribed. Furthermore, direct-to-consumer genetic tests are already available to patients, regardless of whether or not they have been proven to improve care. Despite the

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**Note: The views expressed in this article are those of the author and may not necessarily represent U.S. Food and Drug Administration (FDA) policy. No official endorsement is intended nor should be inferred.*

great promise of personalized medicine, the field is changing very rapidly and exactly how and when tests should be applied clinically is still very much a work in progress. Therefore, this chapter will focus on pharmacogenetic laboratory tests that are FDA-approved, used commonly in clinical practice, or are most likely to be incorporated into clinical practice in the near future.

Presently, organizations such as the National Academy of Clinical Biochemistry (NACB) have established practice guidelines for the application of pharmacogenetics in the practice of laboratory medicine.[7] Coordinately, clinical pharmacology groups such as the Clinical Pharmacogenetics Implementation Consortium (CPIC) have published practice guidelines for specific drug/gene pairs with clinical importance as data become available.[8] Taken together, guidelines from these organizations and others will likely be useful in bringing together the fields of laboratory medicine and clinical pharmacology in the application of pharmacogenetics. An overview of such guidelines and their implications will be discussed.

Pharmacogenetic Testing Versus Disease Genetic Testing

Although laboratory testing for pharmacogenetic and genetic polymorphisms/mutations will yield the same general types of results, the target populations and how the test results are used may be in principle quite different. Clinically used pharmacogenetic tests provide information that may aid in selection or dosing of medications. Therefore, individuals receiving pharmacogenetic tests will typically be candidates for a particular therapeutic agent. Individuals receiving disease genetic tests, on the other hand, will usually be those who are healthy (e.g., for screening purposes), or at risk of developing or are suspected of having a particular disease or condition.

Historically, pharmacogenetic testing has been considered to have fewer ethical issues surrounding it than disease genetic testing.[9] However, while this is still generally considered to be the case, the risks of pharmacogenetic testing also have been outlined and a framework created to ensure appropriate delivery of pharmacogenetic information in the healthcare system.[10] This framework outlines three major considerations regarding whether a particular pharmacogenetic test raises ethical issues: whether the genetic variant is inherited or acquired, whether the goal of testing is to address a specific clinical question or to provide information for future clinical care, and whether the test reveals ancillary clinical information (e.g., disease risk).[10]

Pharmacogenetics and Personalized/Precision Medicine

Pharmacogenetics offers one piece to the puzzle of personalized or precision medicine. Personalized medicine seeks to tailor medical therapy to individual characteristics of patients. It can include genetics information, as in pharmacogenetics, or any other molecular analyses (such as metabolomics, proteomics, etc.). This chapter will focus on pharmacogenetics as a means of providing personalized medicine.

DRUG DISPOSITION-RELATED MOLECULAR TESTS

Pharmacokinetics is concerned with the fate of drugs or other substances once administered and studies the rate and extent of **a**bsorption, **d**istribution, **m**etabolism, and **e**xcretion (ADME). As early as the 1950s, it was noted that a great deal of interpatient variability existed in the pharmacokinetics of many drugs. One common source of interpatient variability occurs in drug metabolism. Drug disposition reactions can be divided into phase I, phase II, and phase III reactions. Phase I reactions typically involve processes such as oxidation, reduction, and hydrolysis of compounds and are typified by hepatic cytochrome P450 (CYP) drug metabolism. Phase II reactions include conjugation or synthetic reactions such as glucuronidation, sulfation, methylation, acetylation, and others. The purpose of phase II metabolism is to make compounds more water-soluble and facilitate excretion. Finally, phase III reactions are characterized by transport protein-mediated cellular efflux of drugs usually at the level of the gut, liver, kidney, and highly sequestered tissues. Genetic variability occurs in each of the above phases of drug disposition (**Figure 7-1**).

Cytochrome P450 System

Although many of the genes encoding CYP enzymes are highly polymorphic, *CYP2D6, CYP2C9,* and *CYP2C19* have genetic variations (polymorphisms), which can describe fairly predictable distributions of drug concentrations, making them clinically relevant for some pharmacogenetic tests. Metabolizer status can be described as extensive (i.e., "normal"), intermediate, poor, or ultrarapid based on the presence or absence of gene variations. This genotype–phenotype relationship could help identify poor metabolizers likely to experience side effects (or therapeutic

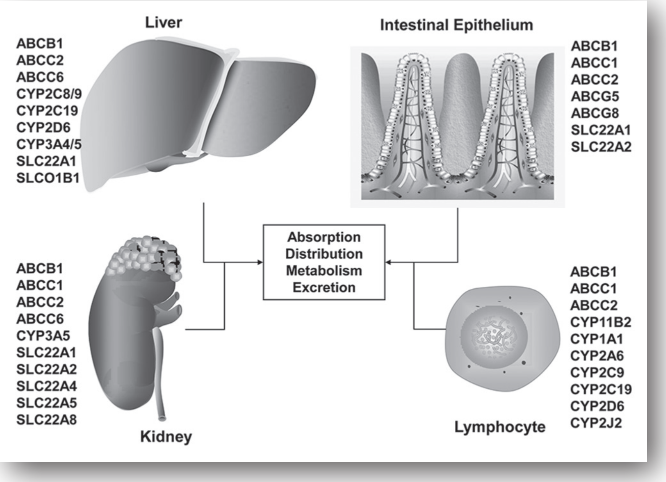

FIGURE 7-1. Sample polymorphic genes involved in drug pharmacokinetics. Polymorphic genes involved in drug pharmacokinetics are listed next to organs involved in drug absorption, distribution, metabolism, and excretion. VisiScience, Inc. software was used in the creation of the image.

failure in the case of prodrugs requiring activation) to usual doses of CYP450-metabolized drugs or ultrarapid metabolizers more prone to therapeutic failure (or toxicity in the case of prodrugs). With knowledge of genotype, drug doses in these individuals with altered metabolism could then be increased or decreased as appropriate or alternative drugs may be chosen. This ability of genotype to predict drug metabolizing phenotype may be especially important for drugs with narrow therapeutic indices and less important for wide therapeutic index drugs.[11] The haplotypes containing the specific polymorphisms leading to these phenotypes are typically described using star (*) nomenclature as defined by the Cytochrome P450 Nomenclature Committee (http://www.cypalleles.ki.se). The *1/*1 genotype is considered the common or normal, fully functioning form of the gene. It is important to note that the *1 allele is reported if all other alleles tested for are absent. Therefore, if rare loss-of-function alleles have not been interrogated, a *1/*1 genotype may erroneously be reported.

CYP2D6

The *CYP2D6* gene contains over 100 alleles, which can lead to a normal-functioning, reduced, or nonfunctional protein, or even multiple copies of the gene. The most common nonfunctional alleles are *3 (rs35742686), *4 (rs1065852, rs3892097, and rs1135840), *5 (gene deletion), and *6 (rs5030655). Multiple copies of the gene are designated by the allele and how many copies are detected (e.g., *1x2). One of the most reproducible associations between *CYP2D6* genotype and a drug response occurs with codeine. Codeine is a prodrug requiring metabolism by CYP2D6 into its active form, morphine, for its analgesic effect. Therefore, individuals who are *CYP2D6* poor metabolizers are at risk of therapeutic failure and those who are ultrarapid metabolizers are at risk of toxicity. Alternative analgesic therapy is recommended in both of these groups of patients.[12] Guidelines have been published with recommendations for *CYP2D6* genetic testing interpretation and suggested clinical action for the test results.[12] **(Minicase 1.)**

MINICASE 1

Codeine Pharmacogenetics

Jennifer P. is a 30-year-old woman who had a caesarean section and delivered a healthy baby boy 12 days ago. She was given codeine in the hospital for pain control and was given a codeine prescription upon discharge. She presents with her baby to the emergency department because her baby has been exhibiting extreme sleepiness, poor feeding, and trouble breathing. She is genotyped and found to be *CYP2D6*1/*1X2.*

QUESTION: Which gene(s) impact response to codeine?

DISCUSSION: Codeine is a prodrug that is activated to morphine by CYP2D6. Other genes that may impact codeine metabolism and response include *UGT2B7,* which is involved in the formation of morphine-6-glucuronide, the *ABCB1* transporter gene, and the opioid receptor µ1 gene *OPRM1.*

QUESTION: Genotypes for *CYP2D6* are represented by what four phenotypes?

DISCUSSION:

1. Normal (also known as *extensive*) metabolizers—WT/WT, WT/reduced, and WT/absent. This phenotype achieves the expected concentrations of morphine.
2. Intermediate metabolizer—reduced/absent. This phenotype has intermediate enzyme activity and reduced morphine formation.
3. Poor metabolizers—absent/absent. This phenotype lacks CYP2D6 enzyme activity and has greatly reduced morphine formation leading to insufficient pain relief when given codeine.
4. Ultrarapid metabolizers—WT/increased, increased/increased. This phenotype has increased enzyme activity and increased morphine formation leading to increased risk of toxicity.

QUESTION: What is an activity score and which score goes with each phenotype from the above question?

DISCUSSION: Activity score is used in addition to the traditional drug metabolizer phenotypes because of the large number of alleles present in *CYP2D6* and the wide range of enzyme activity even within phenotypic groups. To determine an activity score, the combination of alleles is used to determine diplotype. Each functional group is then assigned an activity value ranging from 0 to 1 (e.g., 0 for nonfunctional, 0.5 for reduced function, and 1 for fully functional). If multiple copies of the *CYP2D6* gene are detected, the activity score is multiplied by the number of copies of each allele present. Therefore, a normal metabolizer with an activity score of 1 will have less enzyme activity than a normal metabolizer with an activity score of 2.

- Normal metabolizers—Activity score is 1–2.
- Intermediate metabolizer—Activity score is 0.5.
- Poor metabolizers—Activity score is 0.
- Ultrarapid metabolizers—Activity score is >2.

QUESTION: What do you suspect is going on with this patient's baby, and what treatment management decisions would you recommend based on her genotype?

DISCUSSION: Per the CPIC guidelines, this genotype is an ultrarapid metabolizer with increased enzyme activity (~1–2% of patients). *CYP2D6* ultrarapid metabolizers treated with codeine have rapid intoxication even with low doses due to increased formation of morphine. Codeine is excreted into the breastmilk; therefore, suspect that the baby is receiving toxic levels of morphine. Codeine should be avoided in ultrarapid metabolizers and alternative analgesics considered.

QUESTION: What analgesics are not impacted by *CYP2D6*? What other medications might variants in this gene affect?

DISCUSSION: Analgesics not impacted by *CYP2D6* include morphine and nonopioids. Tramadol, hydrocodone, and, to a lesser extent, oxycodone all have metabolism impacted by *CYP2D6.*

CYP2C19

The *CYP2C19* gene contains over 35 alleles leading to normal-, reduced-, no-, or increased activity. The most common nonfunctional alleles are *2 (rs4244285) and *3 (rs4986893), which account for 85% of reduced function alleles in Caucasians and Africans and 99% of reduced function alleles in Asians. The other reduced or nonfunctional alleles, *4 (rs28399504), *5 (rs56337013), *6 (rs72552267), *7 (rs72558186), *8 (rs41291556), and *10 (rs6413438) are less common. The *17 (rs12248560) allele is a gain-of-function allele and has a frequency of 3–20% depending on ethnicity.[18]

One of the most extensively studied associations between *CYP2C19* polymorphisms and a drug response is with clopidogrel. Clopidogrel is a prodrug requiring activation by two CYP450-dependent steps, both of which involve CYP2C19. Individuals carrying reduced function *CYP2C19* alleles have been shown to have lower active metabolite concentrations, reduced inhibition of platelet aggregation, and increased risk of adverse cardiovascular outcomes when treated with clopidogrel at standard doses compared to those without reduced function alleles.[13-17] Based on these data, the FDA updated the clopidogrel label to indicate that alternative treatment or treatment strategies be considered in individuals with two reduced function *CYP2C19* alleles. Guidelines have been published with treatment recommendations based on *CYP2C19* genotype.[18]

CYP2C9

CYP2C9 contains over 30 alleles that lead to decreased or nonfunctional protein. The most common variants in whites in *CYP2C9* are the *2 (rs1799853) and *3 (rs1057910) alleles, whereas the *5 (rs28371686), *6 (rs9332131), *8 (rs7900194), and *11 (rs28371685) alleles are more prevalent in blacks. The *2 allele has a frequency of approximately 13% in whites, 0% in Asians, and 3% in blacks. The *3 allele has a frequency of approximately 7% in whites, 4% in Asians, and 2% in blacks. One of the most well-documented associations with *CYP2C9* is with warfarin dose requirements (discussed below).[19]

Other CYP450s

CYP3A4 contains over 30 reported polymorphisms, but these variations result in a unimodal distribution of drug clearance, lending themselves less well to use in the clinic setting. Unlike *CYP2D6*, *CYP2C19*, or *CYP2C9*, only a small number of very rare variations cause loss of CYP3A4 activity.[20] A more common single nucleotide polymorphism (SNP), *CYP3A4* *22 (rs35599367), which has an allele frequency of 5–7% in whites has been discovered recently to alter RNA splicing and decrease *CYP3A4* expression in a tissue-specific manner.[21] It has been shown to impact CYP3A substrate drugs in some studies but not others.[20] This unimodal distribution likely results from the small contribution each individual polymorphism in the gene makes to phenotypic variation and the fact that environmental factors may play a bigger role in CYP3A4 activity than with other enzymes. *CYP3A5* has proved to have more predictable associations between polymorphisms and expression of CYP3A5 enzyme. Roughly 10–20% of whites, 85% of blacks, 60% of Hispanics, and 50% of east Asians have genetic variants in *CYP3A5* that cause them to express CYP3A5 hepatically and intestinally.[22] Consequently, this proportion of individuals may require dose modifications of CYP3A5-metabolized drugs. In fact *CYP3A5* genetic variants have been implicated in variable drug responses for many drugs including statins, antiepileptics, calcineurin inhibitors and tacrolimus. Moreover, dosing guidelines based on genotype exist for tacrolimus.[23]

Thiopurine Methyltransferase

Thiopurine methyltransferase (TPMT) is the enzyme responsible for the conversion of azathioprine and 6-mercaptopurine into inactive metabolites. Genetic variants in the *TPMT* gene can result in deficient or absent TPMT activity leading to severe hematological adverse effects with azathioprine or 6-mercaptopurine (6-MP) treatment. The wild-type (common) allele in *TPMT* is designated *TPMT**1. The most common variants in *TPMT* are referred to as *TPMT**2 (rs1800460), *3A (rs1800460 and rs1142345), and *3C (rs1142345) and are derived based on the presence or absence of any of three single nucleotide variants in the gene (G238→C; G460→A; and A719→G). Approximately one in 300 individuals possess two copies of these variant alleles and therefore lack TPMT activity. These individuals require dose reductions of thioguanines like 6-MP on the magnitude of 90% to avoid hematological toxicity. Individuals with one copy of a variant allele make up about 10% of the Caucasian population and require dose reductions of approximately 50%.[24,25]

Azathioprine and 6-MP are used in the treatment of childhood acute lymphoblastic leukemia, rheumatoid arthritis, prevention of renal allograft rejection, and in the management of autoimmune disorders. In 2004, the FDA added language to the package insert of 6-MP indicating that *TPMT* genotyping or phenotyping should be considered prior to treatment.[24] Many major academic cancer hospitals routinely perform TPMT activity testing prior to 6-MP dosing for this indication and practice guidelines have been published.[25] Individuals with two deficient *TPMT* alleles (or deficient activity) require 10-fold starting dose reductions to avoid severe myelosuppression.[25] Individuals with one deficient allele (or intermediate activity) have more variable dose requirements, with 30–60% of heterozygotes being unable to tolerate full doses. Starting dose reductions of 30–50% are recommended in heterozygotes.[25] Thiopurine methyltransferase activity can be determined either by enzymatic testing of red blood cell lysate or by genotyping. A study addressed the prevalence of TPMT enzyme and genotype testing across a national survey of dermatologists, gastroenterologist, and rheumatologists in England.[26] Overall, TPMT enzyme testing was reportedly used by 67% of respondents. This testing was most frequently used by dermatologists (94%), followed by gastroenterologists (60%), and rheumatologists (47%). Genotype testing was not routinely available to practitioners participating in this survey and, hence, was only used by 5% of respondents.[26]

It is important to note that the measurement of TPMT enzyme activity can be impacted by concurrent drugs such as salicylates and by recent blood transfusions. There has been some debate regarding whether genotyping or measurement of enzyme activity is superior.[27] Genotyping may be more useful at the start of therapy to minimize the likelihood of life-threatening toxicity, in the setting of blood transfusion (as phenotyping is inaccurate), and in bowel disease or rheumatoid arthritis where, unlike in leukemia, serial blood draws are not routinely performed. Given the dynamic nature of phenotype compared to genotype, phenotyping may be more useful after a disease flare (as a compliance measure), after several months of therapy (TPMT induction effect has been described), or at the time of an adverse event.

Dihydropyrimidine Dehydrogenase

Dihydropyrimidine dehydrogenase (DPD) metabolizes fluoropyrimidine agents (5-fluorouracil, capecitabine, and tegafur) commonly used in the treatment of solid organ tumors. In the mid-1980s, it was recognized that deficiencies in DPD were heritable and associated with severe 5-fluorouracil toxicity.[28,29] Many polymorphisms in the gene encoding DPD, *DPYD*, have been identified, although very few of them have any effect on enzyme activity. An intronic polymorphism, IVS14 +1 G>A (*DPYD**2A, rs3918290) that results in a splicing defect rendering *DPYD* inactive has been the most widely studied. DPYD*2A is relatively rare with frequencies ranging from than 0.0005% to 3.5% depending on the population.[30] Other polymorphisms, which have been found to reduce DPD activity include *DPYD*3* (rs72549303 G>del), *13 (rs55886062 A>C), and rs67376798 T>A. CPIC guidelines have been published with dosing recommendations based on genotype.[30]

As with TPMT testing, DPD deficiency can be tested for genetically or with enzymatic testing. The clinical performance of using genetic testing to predict toxicity has ranged from 55.3% to 83% for sensitivity, 82% to 100% for specificity (although specificity was not determinable in most studies evaluated), 62% to 100% for positive predictive value, and 82% to 94% for negative predictive value (again, not determinable for most studies).[31,32] Fewer studies have evaluated the test performance characteristics of enzymatic testing, but two studies that did showed that this type of testing had a sensitivity of 60%.[31]

Clinical Significance

When deciding whether drug metabolism polymorphisms might be clinically significant for particular drugs, three main factors should be considered. First, is the drug metabolism enzyme of interest an important route of elimination for the drug in question? If not, even functional polymorphisms in this gene may not have a great impact on the pharmacokinetics of the drug. Second, does the medication of interest have a narrow therapeutic index or steep exposure/response relationship? If not, changes in plasma concentrations may not be great enough to influence the dose-response relationship. Last, are other therapeutic alternatives available to the medication in question? If so, these alternatives may have other routes of metabolism that are not polymorphic, and the variability in pharmacokinetics could be avoided altogether.

DRUG-TARGET-RELATED MOLECULAR TESTS

Germline Variation and Genotype-Guided Therapy

Although pharmacokinetics is concerned with ADME, pharmacodynamics is concerned with drug effects on target molecules, tissues, and physiological processes. There are abundant examples of pharmacogenetic studies in the literature, where the candidate gene of interest was one related to drug target physiology (vis-à-vis drug metabolism).[5] Furthermore, there are illustrative examples in cardiology, oncology, infectious diseases, and others where consideration of genetic variation may improve drug therapy. This section will focus on warfarin. Warfarin is an example where the inclusion of both pharmacokinetic and pharmacodynamic genes improves the ability to predict dose. The next section will focus on illustrative examples of drug-target related molecular tests in oncology.

Vitamin K Epoxide Reductase

Warfarin is the most commonly prescribed anticoagulant for the treatment and prevention of thrombosis. Despite its widespread use, warfarin has a narrow therapeutic range (as measured by the international normalized ratio [INR]) below which thrombosis risk is increased and above which bleeding risk is increased. Although patient-specific factors such as age, sex, race, and diet partially explain variability in warfarin response, these factors don't reliably predict the likelihood of efficacy or bleeding risk. As such, investigators have studied the role of genetic variants in an enzyme responsible for warfarin's metabolism *(CYP2C9)* and target gene vitamin K epoxide reductase complex subunit 1 *(VKORC1)* on warfarin responses. These studies have investigated the impact of genetic and nongenetic factors on endpoints related to INR, bleeding, and clinical efficacy.

CYP2C9 is the major metabolic route for the more potent warfarin enantiomer. The *CYP2C9**1 allele is associated with full metabolic capacity, while the well-studied *2 (rs1799853) and *3 (rs1057910) alleles are associated with decreased metabolic activity, diminished warfarin clearance, and lower warfarin dose requirements.[33-38] By extension, these variant carriers exhibit longer than normal time to achieve target INR and are at increased risk for bleeding.[39] More recently, pharmacogenetic studies also have included analysis of *VKORC1* polymorphisms. Taken in sum, *CYP2C9* and *VKORC1* polymorphisms when considered with clinical correlates of warfarin dose explain approximately 50% of the variability in warfarin dose requirements.[40,41] Dosing algorithms including *CYP2C9*, *VKORC1*, and clinical information are continually being developed and tested, and the FDA updated the warfarin label with estimated doses by genotype.[42-44] Guidelines also have been published with recommendations for clinical interpretation of warfarin pharmacogenetics data.[44]

Warfarin pharmacogenetics has been complicated by contradictory prospective clinical trials assessing pharmacogenetic-guided versus traditional dosing.[45-48] The U.S.-based study was called Clarification of Optimal Anticoagulation Through Genetics (COAG)[46] and the two European studies were called European Pharmacogenetics of Anticoagulant Therapy (EU-PACT).[47,48] All of the studies had the same primary endpoint, time in the therapeutic range. To briefly summarize the trials' findings related to the primary outcome, pharmacogenetic-guided dosing was superior to empiric dosing; however, pharmacogenetic-guided dosing was not superior to a clinical dosing algorithm.[45-48] Further complicating the issue is that in COAG, African Americans did significantly worse with a pharmacogenetic dosing algorithm compared to the clinical algorithm.[46] The pharmacogenetic algorithm used in these studies did not include variants that are associated with warfarin dose requirements in African Americans (*CYP2C9**5, *CYP2C9**6, *CYP2C9**8, *CYP2C9**11, and rs12777823).[49-51] The exclusion of these variants from the algorithm may have overestimated the dose in African-American study subjects.[49] Although time in the therapeutic range (primary endpoint) did not differ between the groups, major bleeding was more common in the clinically guided dosing arm of the COAG trial compared to the genotype-guided dosing arm (HR 0.36; 95% CI 0.15, 0.86; $p = 0.021$).[46]

Tumor Molecular Testing to Guide Therapy Choices

In oncology, the advances in genomic technologies and the realization that many tumors can be subdivided in molecular subsets defined by specific genomic alterations has fueled the development of therapeutic agents targeting these molecular alterations. Therefore, genomic testing to identify patients to be considered for a specific therapy based on the patient's tumor molecular classification is becoming the standard of care. Also, in addition to germline (inherited) variations, tumor cells also can exhibit acquired or noninherited (somatic) variations only present in the tumor tissue. This adds a layer of complexity to molecular testing, involving the acquisition and processing of tumor tissue with adequate quality and quantity to accommodate the test of interest. Moreover, laboratories performing specialized testing should have demonstrated proficiency in the specific technology being used.

It is important to keep in mind that the biological understanding of the molecular landscape of tumors is constantly evolving, as are the genomic technologies used to assess molecular alterations in tumor samples, circulating tumor cells or cell-free tumor DNA. This section deals with five illustrative examples of molecular alterations in tumors at the gene and protein level used to guide therapy: the antihuman epidermal growth factor receptor 2 (anti-HER2) antibody trastuzumab in HER2-positive breast and gastric cancer; the epidermal growth factor receptor tyrosine kinase inhibitors (EGFR TKIs) erlotinib, gefitinib and afatinib in EGFR mutation-positive non-small-cell lung cancer (NSCLC); the anaplastic lymphoma kinase (ALK) inhibitor crizotinib in ALK-positive NSCLC; the serine/threonine-protein kinase B-raf (BRAF) inhibitors vemurafenib and dabrafenib in BRAF mutation-positive melanoma; and the antiprogrammed cell death 1 (anti-PD-1) antibody pembrolizumab in programmed cell death 1 ligand 1 (PD-L1)-positive NSCLC.

HER2-Positive Breast and Gastric Cancer and Anti-HER2 Therapy

Trastuzumab is a monoclonal antibody against the HER2 protein, encoded by the *ERBB2* gene, approved for the treatment of breast cancer. It was noted in the early drug development process that trastuzumab was only effective in a subset of patients. It was subsequently elucidated that trastuzumab is only effective in breast cancers that overexpress the HER2 protein, representing approximately 25% of breast cancer tumors. Pertuzumab, another anti-HER2 monoclonal antibody, was subsequently approved for use in combination with trastuzumab. Consequently, treatment with these agents is predicated on this molecular diagnostic and accurate classification of HER2 tumor status is necessary for optimum treatment of HER2-positive tumors. Although not a test for genetic polymorphisms per se, HER2 testing is performed by methods including immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) to determine the HER2 overexpression and gene amplification, respectively.[53] Both IHC and FISH assays have been approved by the FDA for HER2 testing.[52]

Clinically, tumor biopsies are obtained from individuals with breast cancer in which anti-HER2 therapy may be an option. Immunohistochemistry is often used as initial screening for HER2 overexpression because of its more routine availability in clinical laboratories and lower cost than FISH. Guidelines regarding HER2 testing have recently been updated.[54,55] In one algorithm, a semiquantitative IHC test is performed on the tissue sample to determine the extent of HER2 overexpression. Samples with scores of 0/1+ are considered negative; those with scores of 2+ are borderline/equivocal; 3+ tissues are considered positive.[54,55] In cases of borderline specimens, FISH is recommended for subsequent evaluation with a FISH ratio >2 generally considered a positive test.[54,55]

The test performance characteristics of IHC and FISH have been well described. For example, in analysis of approximately 3000 breast cancer specimens, the positive predictive value of IHC (3+) was 92%, and the negative predictive value (0 or 1+) was 97%.[57] These molecular tests are widely available and used clinically in the setting of breast cancer treatment with trastuzumab. Furthermore, the techniques are not limited to breast tumors and are likely to be extended to other disease states and drug therapies in which gene product expression is a determinant of clinical responsiveness.

More recently, trastuzumab has been approved in combination with chemotherapy as an option for the treatment of patients with HER2-overexpressing metastatic gastric cancer, or gastroesophageal junction (GEJ) adenocarcinoma. Due to differences in tumor histopathology (breast or gastric/GEJ adenocarcinoma), tests developed for the specific tumor type to assess HER2 protein overexpression and HER2 gene amplification should be used.[58]

EGFR Mutation-Positive NSCLC and EGFR TKIs

EGFR aberrant signaling is associated with development and prognosis of certain cancers. Much like HER2-positive breast and gastric cancer dependence on HER2 signaling discussed above, EGFR mutation-positive NSCLC depends on EGFR signaling for proliferation and survival. Therefore, EGFR therapeutic inhibition results in blockage of important processes in the pathogenesis of lung cancers.[59]

In NSCLC, the presence of certain EGFR-activating mutations in the tumor (mostly in exons 18 to 21 of the *EGFR* gene) defines a molecular subset of lung cancer associated with increased sensitivity to EGFR TKIs such as erlotinib, gefitinib, and afatinib in the metastatic setting. The best documented EGFR TKI-sensitizing mutations are exon 19 deletions and L858R in exon 21, representing about 90% of reported EGFR mutations in NSCLC. The remaining 10% of EGFR mutations represent a heterogeneous, less common and less characterized group of mutations. EGFR mutations are most common in East-Asians (35% versus 10% in Whites), female never-smokers, and in patients with adenocarcinoma histology.[60,61] In a 2004 landmark study, Lynch and colleagues identified mutations in the *EGFR* gene in tumors of patients with NSCLC who were responsive to gefitinib.[62] Sensitivity and specificity were 89% and 100%, respectively; positive and negative predictive values were 100% and 88%. Since this publication, several clinical trials have prospectively tested the impact of EGFR mutations on clinical response to EGFR TKIs among lung cancer patients. Results from these studies have underscored the importance of tumor molecular profiling and EGFR mutation testing is currently a standard procedure in guiding therapy choices for advanced NSCLC.[63-65] First-generation (gefitinib, erlotinib) and second-generation (afatinib) EGFR TKIs are FDA-approved for the first-line treatment of patients with metastatic NSCLC whose tumors have EGFR exon 19 deletions or exon 21 (L858R) substitution mutations as detected by an FDA-approved test. These drugs were approved in conjunction with polymerase chain reaction (PCR)-based companion diagnostic tests designed to detect defined EGFR mutations in patients considered for therapy with any of these drugs. **(Minicase 2.)**

ALK-Positive NSCLC and ALK Inhibitors

Crizotinib is a multitargeted receptor tyrosine kinase inhibitor of ALK and other kinases, such as ROS1.[66] Different lung cancer subsets can be molecularly defined by the presence of specific "driver" mutations (e.g., EGFR-activating mutations discussed above), which are key in tumor pathogenesis. These driver molecular alterations are being targeted for therapy, and the prospective testing of lung cancers for various molecular alterations is becoming a new standard of care.[67] One of these unique molecular subsets is characterized by rearrangements involving the *ALK* gene. In NSCLC, the *EML4-ALK* is the most commonly reported *ALK* rearrangement. The resulting fusion gene encodes a cytoplasmic chimeric protein with constitutive kinase activity and tumor promoting potential. *EML4-ALK* occurs in approximately 5% of unselected NSCLC and is more common in never-smokers or light-smokers, in young adult patients, and in patients with adenocarcinomas. Multiple

MINICASE 2

EGFR Mutation Testing in NSCLC

Taylor Z., diagnosed with metastatic NSCLC, has not received prior treatment for metastatic disease. Some of her clinical and demographic characteristics include East-Asian ethnicity, never-smoker status, and tumor with adenocarcinoma histology. These characteristics are known to be associated with a higher frequency of EGFR mutations and with increased sensitivity to EGFR TKIs in NSCLC. Taylor Z. has been treated with an EGFR TKI inhibitor and has had disease progression.

QUESTION: Do you agree on selecting lung cancer patients for first-line treatment with an EGFR TKI solely on the basis of clinical characteristics?

DISCUSSION: EGFR TKIs (e.g., erlotinib, gefitinib and afatinib) are approved as first-line treatment of metastatic NSCLC with specific EGFR mutations (exon 19 deletions or the exon 21 substitution L858R) in conjunction with companion diagnostic tests. Molecular testing is required for appropriate patient selection. This patient was treated with an EGFR TKI based solely on clinical characteristics. Her tumor was not tested for the presence of EGFR mutations or other molecular alterations known to occur in NSCLC, such as ALK rearrangements. In NSCLC, most EGFR mutation-positive tumors are adenocarcinomas or have an adenocarcinoma component. However, not all pulmonary adenocarcinomas harbor EGFR mutations. Also, demographic and clinical characteristics associated with EGFR mutations in NSCLC, such as ethnicity and smoking history are not sensitive enough to select or exclude patients in lieu of molecular testing.[89-91]

This was clearly exemplified by the randomized phase III IRESSA Pan Asia Study (IPASS) of first-line gefitinib versus chemotherapy. In IPASS, patients were selected based on clinical characteristics, which included East-Asian patients with adenocarcinoma that were nonsmokers or former light smokers, regardless of EGFR mutation status. Despite being clinically enriched for the presence of EGFR mutations, retrospective EGFR mutation analyses showed superior benefit of EGFR TKI versus chemotherapy in patients with EGFR mutation-positive tumors but not in patients without EGFR mutations, which highlights the potential risk of selecting patients for EGFR TKI therapy solely based on clinical characteristics.[92,93] Other studies have subsequently supported the role of EGFR mutations in conferring increased sensitivity to EGFR TKIs. Therefore, although EGFR mutations are more common in certain patient subgroups matching some of this patient's demographic and clinical characteristics, molecular testing is still required for adequate selection of patients most likely to benefit from first-line EGFR TKIs.

When considering molecular tests for personalized therapies, it is imperative to follow established guidelines and use appropriate technologies from laboratories with expertise in performing and interpreting the results to provide accurate test results. Also, the interpretation of test results must be made within the context of the patient's clinical history by a qualified professional.

distinct *EML4-ALK* chimeric variants, as well as other fusion partners have been reported.[68]

Crizotinib was developed along with a companion diagnostic and is indicated for metastatic NSCLC with *ALK* rearrangements, as detected by an FDA-approved test. Therefore, the detection of *ALK* rearrangements in NSCLC is necessary for selection of patients for treatment with crizotinib. The companion diagnostic test originally approved in conjunction with crizotinib is a FISH break-apart assay. This assay employs one probe 5' of the *ALK* locus and one probe within the *ALK* gene. When the probe set is hybridized against normal nuclei, it generates a merged (green-red fluorescent) signal that can be visualized microscopically. However, the signal is split when the probe set is hybridized against nuclei with a rearrangement involving the 5' portion of the *ALK* locus. To be considered positive for *ALK* rearrangements and eligible for treatment with crizotinib, at least 15% of the tumor cells analyzed have to harbor either the break-apart signals or have a single 3' *ALK* (red) signal, 50 cells or more have to be counted, and the separation between the 5' and 3' *ALK* probes has to be at least a two signal diameter.[69,70] Other tests are being developed as alternatives to FISH, including IHC and reverse transcription polymerase chain reaction (RT-PCR), which has the potential of identifying fusion variants missed by FISH.[71,72] More recently, an IHC assay was also FDA- approved as a companion diagnostic to select ALK-positive NSCLC patients most likely to benefit from crizotinib. Also recently, two other ALK inhibitors, ceritinib and alectinib, were FDA-approved for the treatment of patients with ALK-positive metastatic NSCLC who have progressed on or are intolerant to crizotinib.

BRAF Mutation-Positive Melanoma and BRAF Pathway Inhibitors

The presence of certain *BRAF* mutations defines a molecular subset of melanoma especially sensitive to inhibition by BRAF inhibitors as single agents or in combination with MEK inhibitors. Vemurafenib and dabrafenib are BRAF inhibitors indicated for the treatment of patients with unresectable or metastatic melanoma with BRAF V600E mutation as detected by an FDA-approved test. Mutated BRAF proteins often have elevated kinase activity leading to aberrant activation of survival and antiapoptotic signaling pathways in the tumor cells.[73] Approximately 40–60% of cutaneous melanomas are positive for BRAF V600 mutations. Among these, the V600E mutations constitute 80–90% of reported V600 BRAF mutations, but other less common BRAF mutations, such as V600K and V600D, also occur. Their role in conferring sensitivity to BRAF inhibitors, however, is not as well documented.[74]

As with other therapeutic agents targeting specific tumor molecular alterations, vemurafenib and dabrafenib also were developed along with PCR-based companion diagnostic tests designed to identify specific BRAF V600 mutations.[75] Currently, BRAF inhibitors are not indicated for patients with wild-type BRAF melanoma due to the potential of paradoxical activation of the mitogen-activated protein kinase (MAPK) pathway and tumor promotion in certain cellular contexts, which highlights the importance of understanding test performance for determining therapy eligibility and the consequences of false-positive and false-negative results.[76]

PD-L1 Expression and Immune Checkpoint Antibodies

PD-1 and its natural ligand, PD-L1, are part of a complex network of immune co-inhibitory signals referred to as immune checkpoints that help modulate the immune response by regulating T cell function. Some tumors escape immune surveillance by expressing PD-L1 and inhibiting T cell–mediated tumor clearance through PD-1/PD-L1 interactions.[77]

Several therapeutic monoclonal antibodies against PD-1 or PD-L1 are being developed to block the PD-1/PD-L1 interaction in different types of cancer as an attempt to enhance antitumor responses by the immune system. Two of these antibodies have been recently FDA-approved for NSCLC. Pembrolizumab is an anti-PD-1 antibody indicated for the treatment of patients with metastatic NSCLC whose tumors express PD-L1 as determined by an FDA-approved test with disease progression on or after platinum-containing chemotherapy. Pembrolizumab was approved in parallel with a PD-L1 IHC companion diagnostic used to determine PD-L1 protein expression levels in tumors of patients considered for treatment.[78] Nivolumab, another anti-PD-1 antibody, was initially approved for second-line squamous NSCLC and more recently the indication was expanded to include both squamous and nonsquamous NSCLC.[79]

Unlike pembrozilumab, nivolumab is indicated for second-line NSCLC regardless of PD-L1 expression. However, based on a prespecified subgroup analyses in a clinical study of patients with metastatic nonsquamous NSCLC, those with higher levels of PD-L1 protein expression in their tumors (as assessed by IHC) appeared to have increased clinical benefit from nivolumab.[78,79] The PD-L1 IHC test was FDA-approved for nonsquamous NSCLC as a complementary diagnostic. Of note, although both diagnostic tests to measure PD-L1 expression are IHC assays, they differ on cutoff definition for PD-L1 positivity among other characteristics. IHC assays to assess PD-L1 expression have proven to be cumbersome due to both technical and biological aspects. As these therapies reach routine practice, efforts to harmonize and standardize testing for PD-L1 by IHC in NSCLC are underway.[80] Of relevance, patients with EGFR or ALK genomic tumor aberrations should have disease progression on FDA-approved therapy for these aberrations prior to receiving pembrolizumab or nivolumab, which underscores the importance of tumor genomic analysis and accurate molecular classification to guide treatment choices.[78,79]

Resistance to Targeted Therapy

Only a percentage of patients respond to targeted therapies. Moreover, despite initial clinical benefit, responders often develop resistance. Elucidating underlying mechanisms of primary or acquired resistance at a molecular level is an intense area of research in oncology. For example, somatic point mutations in the *KRAS* gene, most commonly found in codons 12 and 13, have been strongly associated with primary resistance to the anti-EGFR monoclonal antibodies panitumumab and cetuximab in colorectal cancer. Therefore, these antibodies are not indicated for colorectal cancer patients with tumors positive for these mutations. Of note, different *KRAS* mutations may not predict to the same extent resistance to anti-EGFR antibodies.[81] Point mutations, gene amplifications, changes in protein expression and activation of alternate pathways are among the mechanisms implicated in resistance.[82] Molecular assays to identify these alterations and evaluate their clinical significance are in different stages of development. This is illustrated by osimertinib, which is a new third-generation irreversible EGFR TKI recently approved in conjunction with a PCR-based companion diagnostic assay for metastatic EGFR T790M mutation-positive NSCLC patients who have progressed on or after EGFR TKI therapy. The EGFR T790M second-site mutation, occurring within EGFR exon 20, accounts for approximately 50% of the reported cases of acquired resistance to the reversible EGFR TKIs erlotinib and gefitinib. It also confers decreased sensitivity to the irreversible EGFR TKI afatinib. Many other mechanisms of resistance to EGFR TKIs involving different molecular alterations have been reported in NSCLC and molecular testing prior to receiving osimertinib is required to confirm the presence of the EGFR T790M mutation.[83]

IMMUNE-RELATED ADVERSE REACTIONS AND MOLECULAR TESTS

Variants in immune-related genes are increasingly being associated with drug-induced adverse events. One of the most noted examples is that of abacavir. Immune-mediated hypersensitivity reactions occur in 5–8% of abacavir-treated patients, usually within the first six weeks of treatment. Retrospective case-control studies identified the major histocompatibility complex (MHC) class I region as being associated with this hypersensitivity reaction.[84] Subsequent, prospective randomized-controlled trials demonstrated that screening for the *HLA-B**5701 allele eliminated immunologically confirmed hypersensitivity reactions with a negative predictive value of 100% and a positive predictive value of 47.9%.[85] Current HIV treatment guidelines recommend screening for *HLA-B**5701 prior to the initiation of an abacavir-containing treatment regimen.[86]

Since the association between HLA genotype and abacavir hypersensitivity reaction was identified, severe adverse effects with many other drugs have been noted to be associated with the MHC regions as well. Some examples of these associations are phenytoin-induced cutaneous reactions and *HLA-B**1502, carbamazepine-induced cutaneous reactions and *HLA-B**1502 as well as *HLA-A**3101, flucloxacillin (not currently available in the United States)-induced liver injury and *HLA-B**5701, amoxicillin-clavulanate-induced liver injury and *HLA-A**0201 and *HLA-DRB1**1501-*DQB1**0602, lumiracoxib (not currently available in the United States)-induced liver injury and *HLA-DQA1**0102, and allopurinol-induced cutaneous reactions and *HLA-B**5801.[87,88]

GENOTYPING PLATFORMS

Many commercial genetic tests are available for pharmacogenetic-related genes or gene panels. The AmpliChip (Roche Molecular Diagnostics, Basel, Switzerland) microarray is one such example that is FDA-cleared. It provides analysis for *CYP2D6* and *CYP2C19* genotypes to predict enzymatic activities. The assay tests for up to 33 *CYP2D6* alleles, including gene duplications, and three *CYP2C19* variants and includes software to predict the drug metabolism phenotype based on the combination of alleles present (e.g., normal, intermediate, poor, and ultrarapid metabolizers). Luminex xTAG CYP2D6 Kit (Luminex Corporation, Austin, TX) is another FDA-cleared test that tests for 15 *CYP2D6* alleles as well as gene duplications. Given that commercially available genetic testing options change frequently, we will not discuss all available tests. However, the Genetic Testing Registry provides a central location for voluntary submission of genetic test information by providers. (www.ncbi.nlm.nih.gov/gtr/)

Drug metabolism genotyping assays do have two limitations with which the clinician should be familiar: (1) new alleles that alter metabolic function are constantly being discovered, so there are patients who will not be perfectly assigned to a drug metabolism group or could be inappropriately assigned the *1/*1 genotype by default because these alleles are untested; and (2) because genotyping does not directly measure metabolic activity or drug concentrations, the effect of drug interactions on the drug metabolizing phenotype are not captured by the test. In other words, a person may genotypically be a normal metabolizer but phenotypically be a poor metabolizer because they are taking a drug that inhibits the particular CYP450 enzyme. This limitation highlights the importance of proper patient-specific interpretation of CYP genotyping results in clinical practice.

To accommodate the growing number of genetic variants of (potential) clinical significance, clinical testing is moving from single variants to multiplexed panels that can simultaneously interrogate a limited number of variants (polymorphisms and hotspot mutations). Next-generation sequencing (NGS) technologies, although not yet implemented in routine practice, provide even more comprehensive genomic analysis, including genome copy number changes and structural rearrangements, which are not captured by multiplexed panels. NGS technologies can perform whole-genome sequencing, targeted sequencing including whole exome sequencing, transcriptome analysis, and epigenetic profiling. NGS-based approaches have been used to uncover underlying disease biology and to identify markers influencing response to therapies. In the context of clinical oncology, high-throughput gene sequencing has the potential to change patient care from diagnosis to disease management. However, the integration and accurate biological interpretation of the large genomic data generated by these platforms is still a major challenge for clinical implementation.[94-96]

The cost of pharmacogenetic testing varies depending on the number of alleles being tested. Although multigene arrays are more cost-effective than genotyping individual SNPs, they currently do not have Current Procedural Terminology codes for reimbursement; thus, genotyping for clinical care is often done as individual tests.[97] The turnaround time also varies but is usually 24–96 hours. In institutions where the clinical laboratory is onsite, turnaround could be as fast as four hours depending on the assay. Generally, genotyping results that are to be used in the clinical care of patients need to be generated from certified laboratories (such as those approved or certified by the Clinical Laboratory Improvements Amendments [CLIA] or the College of Pathologists [CAP]). Germline variation is stable throughout a person's lifetime; therefore, in general, it only needs to be done once. However, it is important to note which alleles are tested because additional alleles may have been discovered, which will require additional testing to gain information on those alleles.

Test Performance and Decision Making

Laboratory testing and clinical decision making using pharmacogenetics can be incorporated into the total testing process (TTP) as outlined by Schumacher and Barr.[98] The TTP is divided into the preanalytical, analytical, and postanalytical phases and is designed to systematically improve patient care by asking a patient-oriented question; determining and ordering the appropriate test to answer the question; collecting and processing the sample; performing the test; reporting and interpreting the results; and taking clinical action to positively impact the patient.

The TTP is well established in therapeutic drug monitoring using serum drug concentrations, where the appropriate test (e.g., free or total drug concentration) is ordered; the phlebotomist draws the appropriate sample (e.g., steady-state peak or trough); the results are reported and interpreted in the context of the patient's status (e.g., exhibiting signs of toxicity); and a clinical decision is made (e.g., dosage change or drug discontinuation). The same TTP can be applied to pharmacogenetics when single genes have a large impact on drug response (often pharmacokinetics). These drug/gene pairs with high level supporting data are often the subject of CPIC guidelines.

Perhaps the biggest barrier to application of pharmacogenetics is the inability to apply genotype results. This would require integration into the electronic health record and adequate clinical decision support to guide practitioners in the use of pharmacogenetic data. In this regard, the TTP as applied to pharmacogenetics could be interdisciplinary, involving physicians, translational scientists, clinical pharmacists, and others. Additionally, accreditation standards such as those put forth by The Joint Commission, CLIA, CAP and others will have to be addressed when formally incorporating pharmacogenetic testing into institution-based practice.

As mentioned elsewhere in the chapter, organizations including the NACB and CPIC are working in a multidisciplinary fashion to address issues related to pharmacogenetic test methodology; standardization and quality control/assurance of tests; selection of appropriate test panels; reporting and interpretation of results; and other issues related to testing applied in clinical practice.[7,8] Although expansive in its scope, the NACB guidelines specifically highlight the role of the clinical laboratory in development of genotyping strategies that maximize test performance (i.e., sensitivity and specificity) for clinical application. Furthermore, the guideline recommendations developed the following criteria for a pharmacogenetic test to be clinically useful: (1) *analytical reliability* (consistent measurement of the genotype/allele tested); (2) *operational implementation* (operational characteristics should not be beyond the complexity level certified by CLIA for reference laboratories); (3) c*linical predictive power (*specificity and sensitivity consistent with other diagnostics in use); and (4) *compatibility with therapeutic management* (interpretation of genotype results should inform clinical decision making). Interestingly, model examples outlined by the guidelines for drugs in which pharmacogenetics can be implemented include warfarin (*CYP2C9* and *VKORC1*) and irinotecan (*UGT1A1*). The CPIC has taken the approach of publishing clinical practice guidelines for specific drug/gene pairs as enough data become available to warrant clinical action based on genotype. A sample of drug/gene pairs that contain pharmacogenetic information, which can inform dosing or patient selection, is shown in **Table 7-1**.

SUMMARY

Pharmacogenetics is currently being used most widely in hematology/oncology and holds the promise of improving patient care by adding another dimension to therapeutic drug monitoring in other diseases. The use of genetic information will likely be applied to chronic drug therapy for agents with narrow therapeutic indices such as warfarin. The field of pharmacogenetics is evolving rapidly. Consequently, specific information regarding molecular tests and labeling information are likely to constantly change. Basic skills in interpreting genetic information will serve as an important foundation for laboratory medicine and drug therapy as more clinical applications of pharmacogenetics emerge.

For pharmacogenetics to translate to practice, the research and clinical communities jointly must create a meaningful level of evidence in support of pharmacogenetics-enhanced, therapeutic decision making. Because of their unique training and position in the healthcare sector, pharmacists can foresee the forefront of pharmacogenetics research and application. Pharmacists will likely be called upon to synthesize evidence-based practices for incorporating genetic information into treatment algorithms. Once a genetic biomarker is validated (e.g., thiopurine pharmacogenetics), clinicians (including pharmacists) will be responsible for appropriate use and interpretation of the genetic test. The pharmacist's drug and disease expertise, coupled with an understanding of pharmacogenetic principles, may lead to a revolutionary treatment paradigm with enhanced patient outcomes as the ultimate goal.

TABLE 7-1. Drugs with Actionable Dosing/Patient Selection Guidelines Based on Germline Variation[a]

DRUG	GENE(S)	GUIDELINES
Abacavir	*HLA-B*	CPIC, DPWG
Acenocoumarol	*CYP2C9 and VKORC1*	DPWG
Allopurinol	*HLA-B*	CPIC, American College of Rheumatology
Amitriptyline	*CYP2C19 and CYP2D6*	CPIC, DPWG
Aripiprazole	*CYP2D6*	DPWG
Atazanavir	*UGT1A1*	CPIC
Atomoxetine	*CYP2D6*	DPWG
Azathioprine	*TPMT*	CPIC, DPWG
Boceprevir	*IFNL3*	CPIC
Capecitabine	*DPYD*	CPIC, DPWG
Carbamazepine	*HLA-B and HLA-A*	CPIC, Canadian Pharmacogenomics Network for Drug Safety,
Carvedilol	*CYP2D6*	DPWG
Citalopram	*CYP2C19*	CPIC, DPWG
Clomipramine	*CYP2C19 and CYP2D6*	CPIC, DPWG
Clopidogrel	*CYP2C19*	CPIC, DPWG
Clozapine	*CYP2D6*	DPWG
Codeine	*CYP2D6*	CPIC, DPWG, Canadian Pharmacogenomics Network for Drug Safety
Desipramine	*CYP2D6*	CPIC
Doxepin	*CYP2C19 and CYP2D6*	CPIC, DPWG
Duloxetine	*CYP2D6*	DPWG
Escitalopram	*CYP2C19*	CPIC, DPWG
Esomeprazole	*CYP2C19*	DPWG
Flecainide	*CYP2D6*	DPWG
Fluorouracil	*DPYD*	CPIC, DPWG
Flupenthixol	*CYP2D6*	DPWG
Fluvoxamine	*CYP2D6*	CPIC
Glibenclamide	*CYP2C9*	DPWG
Glicazide	*CYP2C9*	DPWG
Glimepiride	*CYP2C9*	DPWG
Haloperidol	*CYP2D6*	DPWG
Hormone contraceptives	*F5*	DPWG
Imipramine	*CYP2C19 and CYP2D6*	CPIC, DPWG
Irinotecan	*UGT1A1*	DPWG, French joint working group
Ivacaftor	*CFTR*	CPIC
Lansoprazole	*CYP2C19*	DPWG
Mercaptopurine	*TPMT*	CPIC, DPWG
Metoprolol	*CYP2D6*	DPWG
Mirtazapine	*CYP2D6*	DPWG
Moclobemide	*CYP2C19*	DPWG
Nortriptyline	*CYP2D6*	CPIC, DPWG
Olanzapine	*CYP2D6*	DPWG
Olaparib	*BRCA*	NCCN Guideline
Omeprazole	*CYP2C19*	DPWG
Oxycodone	*CYP2D6*	DPWG
Pantoprazole	*CYP2C19*	DPWG
Paroxetine	*CYP2D6*	CPIC, DPWG
Peginterferon-2a	*IFNL3*	CPIC
Peginterferon-2b	*IFNL3*	CPIC
Phenprocoumon	*CYP2C9 and VKORC1*	DPWG
Phenytoin	*CYP2C9 and HLA-B*	CPIC, DPWG
Propafenone	*CYP2D6*	DPWG
Rabeprazole	*CYP2C19*	DPWG
Rasburicase	*G6PD*	CPIC
Ribavirin	*IFNL3*	CPIC, DPWG
Risperidone	*CYP2D6*	DPWG
Sertraline	*CYP2C19*	CPIC, DPWG
Simvastatin	*SLC01B1*	CPIC
Tacrolimus	*CYP3A5*	CPIC, DPWG
Tegafur	*DPYD*	CPIC, DPWG
Telaprevir	*IFNL3*	CPIC
Thioguanine	*TPMT*	CPIC, DPWG
Tolbutamide	*CYP2C9*	DPWG
Tramadol	*CYP2D6*	DPWG
Trimipramine	*CYP2C19, CYP2D6*	CPIC
Venlafaxine	*CYP2D6*	DPWG
Voriconazole	*CYP2C19*	DPWG, CPIC
Warfarin	*CYP2C9, VKORC1*	CPIC, DPWG
Zuclopenthixol	*CYP2D6*	DPWG

CPIC = Clinical Pharmacogenetics Implementation Consortium; DPWG = Dutch Pharmacogenetics Working Group; NCCN = National Comprehensive Cancer Network.

[a] Based on a table from PharmGKB.org; not necessarily an all-inclusive list.

LEARNING POINTS

1. ***Describe the types of genes that could impact drug response.***

   ANSWER: Genes that influence the pharmacokinetics (e.g., drug metabolism enzymes or drug transporters) or pharmacodynamics (e.g., drug targets) of a drug could impact drug response. Genetic variation that impacts pharmacogenetics could be germline (i.e., host DNA) or somatic (e.g., tumor DNA).

2. ***How might pharmacogenomics enhance therapeutic drug monitoring?***

   ANSWER: Traditional patient-specific factors such as age, sex, renal function, hepatic function, and body weight are frequently used to determine appropriateness of a particular drug or dose for an individual. However, these factors only partially account for the likelihood of efficacy or toxicity. As our knowledge of how genetic variability impacts drug response is solidified, we can begin to incorporate pharmacogenetic information into algorithms for optimizing pharmacotherapy for individual patients and move toward personalized medicine.

3. ***Who is best equipped to incorporate pharmacogenomics into clinical decision making?***

   ANSWER: The incorporation of pharmacogenomics will require an interdisciplinary team of healthcare providers with knowledge of the specific pharmacological properties of individual drugs, molecular biology, genetics, laboratory medicine, clinical medicine, genetic counseling, and economics. In addition, patients and consumers will likely be active participants and drivers of the use of genetic tests in clinical practice.

4. ***Where can pharmacogenomics information be obtained?***

   ANSWER: Many drug/gene pairs with actionable pharmacogenetics information have practice guidelines published by CPIC and updated on the Pharmacogenomics Knowledge Base website (www.pharmgkb.com). This website also contains other valuable pharmacogenomics information even when guidelines are not yet available. A great deal of pharmacogenomics information is not yet actionable and can be found in the primary literature. Relevant pharmacogenomics information also can be communicated through product labeling, although this information may not be sufficient by itself to guide clinical decision making because patient-specific factors also should be taken into account. For example, the clopidogrel label has incorporated pharmacogenomics information on CYP2C19 in a boxed warning that states, "…poor metabolizers treated with Plavix at recommended doses exhibit higher cardiovascular event rates following ACS or PCI than patients with normal CYP2C19 function. Tests are available to identify a patient's CYP2C19 genotype and can be used as an aid in determining therapeutic strategy. Consider alternative treatment or treatment strategies in patients identified as CYP2C19 poor metabolizers." However, practical recommendations for when genotyping should occur and specific dose adjustments are not provided and must be determined as part of the clinical decision-making process. Therefore, the primary literature would also have to be consulted and interpreted.

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PART II

SYSTEM DISORDERS AND DIAGNOSTIC TESTS

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THE HEART: LABORATORY TESTS AND DIAGNOSTIC PROCEDURES

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OBJECTIVES

After completing this chapter, the reader should be able to

- Describe the normal physiology of the heart
- Describe the electrocardiogram changes reflected by myocardial ischemia and infarction
- Explain the roles of the different biochemical markers in the diagnosis of coronary artery disease, acute coronary syndrome, and heart failure
- Given a patient's history, clinical presentation, cardiac biochemical markers, and electrocardiographic findings, assess the presence and type of acute coronary syndrome
- Given a patient case, assess the presence and type of heart failure
- Describe the role of pharmacologic agents in noninvasive imaging studies
- Describe other diagnostic procedures used for the evaluation of coronary artery disease, acute coronary syndrome, and heart failure

The heart has two basic properties: electrical and mechanical. The two work in harmony to propel blood, delivering oxygen and nutrients to all body tissues. Heart cells responsible for these properties are (1) pacemaker cells or the "electrical power" of the heart; (2) electrical conducting cells or the "hardwiring circuitry" of the heart; and (3) myocardial cells or the contractile units of the heart. Disturbances in the electrical system result in rhythm disorders, also known as *arrhythmias* or *dysrhythmias*. The pumping action is accomplished by means of striated cardiac muscle, which largely composes the myocardium. A number of cardiovascular diseases disrupt the mechanical function of the heart including coronary artery disease (CAD), acute coronary syndrome (ACS), and heart failure.

The management of ACS, heart failure, and potential complications of each contribute greatly to the overall health of and cost incurred by society. Laboratory tests are essential for establishing the diagnosis and determining the prognosis of patients. Accurate and expeditious assessment of a patient presenting with symptoms suggestive of ACS guides individualized treatment to optimize a patient's short-term and long-term outcomes. Conversely, rapid exclusion of the diagnosis permits early discharge from the coronary care unit or hospital. Laboratory and other diagnostic tests used in evaluating the patient with possible ACS and heart failure are discussed in this chapter.

CARDIAC PHYSIOLOGY

The heart consists of two pumping units that operate in parallel, one on the right side and the other on the left side. Each is composed of an upper chamber, the atrium, and a lower chamber, the ventricle. The atrium serves as a passive portal to the ventricle and is a weak pump that helps move blood into the ventricle. The atrial contraction or kick is responsible for 20–30% of ventricular filling. The right and left ventricles supply the primary force that propels blood through the pulmonary and peripheral circulation, respectively (**Figure 8-1**).

The functional unit of the heart is comprised of a network of noncontractile cells that form the conduction system, which is responsible for originating and conducting action potentials from the atria to the ventricles. This leads to the excitation and contraction of the cardiac muscle, which is responsible for the pumping of the blood to the other organs.

The normal adult human heart contracts rhythmically at approximately 70 beats per minute (bpm). Each cardiac cycle is divided into a systolic and diastolic phase. During each cycle, blood from the systemic circulation is returned to the heart via the veins, and blood empties from the superior and inferior vena cavae into the right atrium. During the diastolic phase, blood passively fills the right ventricle through the tricuspid valve with an active filling phase by atrial contraction just prior to end-diastole. During systole, blood is then pumped from the right ventricle through the pulmonary artery to the lungs where carbon dioxide is removed and the blood is oxygenated. From the lungs, blood returns to the heart via the pulmonary veins and empties into the left atrium. Again, during diastole, blood empties from the left atrium through the mitral valve into the main pumping chamber,

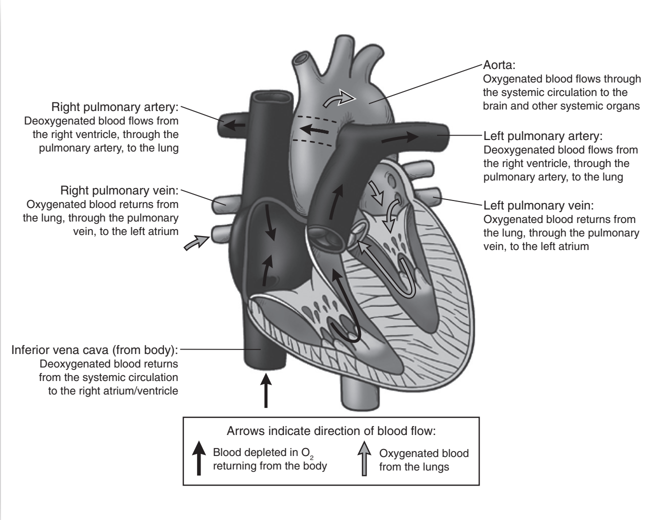
Right pulmonary artery:
Deoxygenated blood flows from the right ventricle, through the pulmonary artery, to the lung
Right pulmonary vein:
Oxygenated blood returns from the lung, through the pulmonary vein, to the left atrium
Inferior vena cava (from body):
Deoxygenated blood returns from the systemic circulation to the right atrium/ventricle
Aorta:
Oxygenated blood flows through the systemic circulation to the brain and other systemic organs
Left pulmonary artery:
Deoxygenated blood flows from the right ventricle, through the pulmonary artery, to the lung
Left pulmonary vein:
Oxygenated blood returns from the lung, through the pulmonary vein, to the left atrium
Arrows indicate direction of blood flow:
Blood depleted in O2 returning from the body
Oxygenated blood from the lungs

FIGURE 8-1. Blood flow through the heart and the pulmonary and systemic circulation.

the left ventricle. With systole the left ventricle contracts and blood is forcefully propelled into the peripheral circulation via the aorta (Figure 8-1). At rest, the normal heart pumps approximately 4–6 L of blood per minute. Maintaining normal cardiac output (CO) is dependent on the heart rate (HR) and stroke volume (SV).

$$\text{CO (mL/min)} = \text{HR (beats/min)} \times \text{SV (mL/beat)}$$

The SV, defined as the volume of blood ejected during systole, is determined by intrinsic and extrinsic factors including myocardial contractility, preload, and afterload. The coronary arteries, the arteries supplying the heart muscle, branch from the aorta just beyond the aortic valve and are filled with blood primarily during diastole. The major coronary arteries are depicted in **Figure 8-2**. In the face of increased myocardial metabolic needs, the heart is able to increase coronary blood flow by vasodilation to meet myocardial oxygen demand.

CARDIAC DYSFUNCTION

Decreased CO compromises tissue perfusion, and depending on the severity and duration, may lead to significant acute and chronic complications. A number of cardiac conditions lead to decreased CO, including hypertensive heart diseases, heart failure, valvular heart diseases, congenital heart diseases, diseases of the myocardium, conduction abnormalities, CAD, and ACS. This chapter focuses on the various tests used in the diagnosis and assessment of patients presenting with CAD, ACS, and heart failure.

Also known as *ischemic heart disease* (IHD), CAD is caused by atherosclerosis of the coronary arteries, resulting in lumen narrowing and blood flow reduction to the myocardium perfused by the affected artery. This leads to tissue ischemia and chest pain. Severe reduction in or total interruption of blood flow may lead to severe tissue ischemia or infarction, resulting in a clinical presentation as a type of ACS.

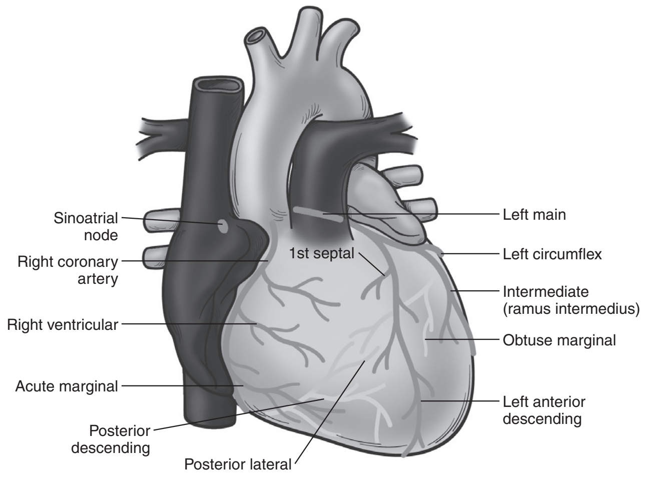
Sinoatrial node
Right coronary artery
Right ventricular
Acute marginal
Posterior descending
Posterior lateral
1st septal
Left main
Left circumflex
Intermediate (ramus intermedius)
Obtuse marginal
Left anterior descending

FIGURE 8-2. Major coronary arteries and their principal branches.

Patients with severe symptoms of myocardial ischemia or acute myocardial infarction (MI) may be experiencing one of three types of ACS: unstable angina (UA), non-ST-segment elevation MI (NSTEMI), or ST-segment elevation MI (STEMI). The most common cause for ACS is atherosclerotic plaque rupture and subsequent obstruction of the coronary lumen by thrombosis composed of platelet aggregates, fibrin, and entrapped blood cells leading to myocardial ischemia. When a coronary artery is occluded, the location, extent, rate, and duration of occlusion determine the severity of myocardial ischemia resulting in UA, NSTEMI, or STEMI.

According to the universal definition, MI may be classified into different types[1]:

- **Type 1:** Spontaneous MI related to ischemia due to a primary coronary event such as plaque erosion and rupture, fissuring, or dissection
- **Type 2:** MI secondary to ischemia due to an imbalance between oxygen supply and demand, such as in coronary artery spasm, coronary embolism, anemia, arrhythmias, hypertension, or hypotension
- **Type 3:** MI resulting in sudden cardiac death in the setting of absent biomarkers
- **Type 4a:** MI associated with percutaneous coronary intervention (PCI)
- **Type 4b:** MI associated with stent thrombosis as documented by angiography or at autopsy
- **Type 5:** MI associated with coronary artery bypass graft

Complications of MI include cardiogenic shock, heart failure, ventricular and atrial arrhythmias, ventricular rupture or ventricular septal defect formation, cardiac tamponade, pericarditis, papillary muscle rupture, mitral regurgitation, and embolism. Initial assessment of the patient presenting with ACS may be confounded by the presence and severity of the above complications.

Heart failure is a syndrome in which the heart is unable to pump blood at a rate sufficient to meet the demands of the body or unable to accept the fluid volume with which it is presented. Heart failure is classified based on left ventricular ejection fraction (LVEF) and indirect measurement of the contractility of the left ventricle. Heart failure is currently defined as either heart failure with reduced ejection fraction (HF*r*EF)—also referred to as *systolic heart failure* when LVEF is ≤40%—or heart failure with preserved ejection fraction (HF*p*EF)—also referred to as *diastolic heart failure* when LVEF is ≥50%. Patients falling in an intermediate group with LVEF between 41% and 49% are classified as having HF*p*EF, borderline. Patients with current LVEF >40% and a history of HF*r*EF in the past are classified as having HF*p*EF, improved.[2]

Common etiologies for heart failure include atherosclerotic disease, valvular diseases, and hypertension. Signs and symptoms consistent with heart failure may be attributed to volume overload and congestion (e.g., elevated jugular venous pressure, peripheral edema, pulmonary congestion and edema, and dyspnea) and hypoperfusion (e.g., tachycardia (HR >100 bpm), cold extremities, cyanosis, and fatigue).

ELECTROCARDIOGRAPHY

Electrocardiography is the recording of the electrical activity of the heart on an electrocardiogram (ECG).

Normal Conduction System and Electrocardiogram Recording

The conduction system is composed of specialized noncontractile cells that serve to originate and conduct action potentials in the appropriate sequence and at an appropriate rate from the atria to the ventricles. At rest, the cardiac cells are more negatively charged intracellularly than extracellularly, or polarized, with a voltage difference of 60–90 mV. When excited, ionic currents across cell membranes lead to charge shifting where the interior of the cells become more positive (depolarization) and an action potential is generated. Calcium influx leads to the excitation–contraction coupling of the cells. Subsequently, the action potential is propagated and the cells return to a normal resting state (repolarization). *Depolarization* is the electrical phenomenon that leads to myocardial contraction, and *repolarization* is the electrical phenomenon that leads to myocardial relaxation. The ECG provides a pictorial presentation of the depolarization and repolarization of atrial and ventricular cells that can be assessed by reviewing a number of waves and intervals.

Normally, an electrical impulse originates in the sinoatrial node and is propagated through Bachmann bundle and internodal tracts, the atrioventricular node, bundle of His, the left and right bundle branches, and the Purkinje fibers resulting in one cardiac cycle. Each cardiac cycle is presented on ECG by the P wave reflecting atrial depolarization, the QRS complex reflecting ventricular depolarization, and the T wave reflecting ventricular repolarization (**Figure 8-3**). By placing multiple leads on the patient, the electrical impulses of the heart are recorded from different views. The standard ECG is composed of 12 leads: six limb leads (I, II, III, AVR, AVL, and AVF) and six chest leads (V_1–V_6). Different leads provide specific

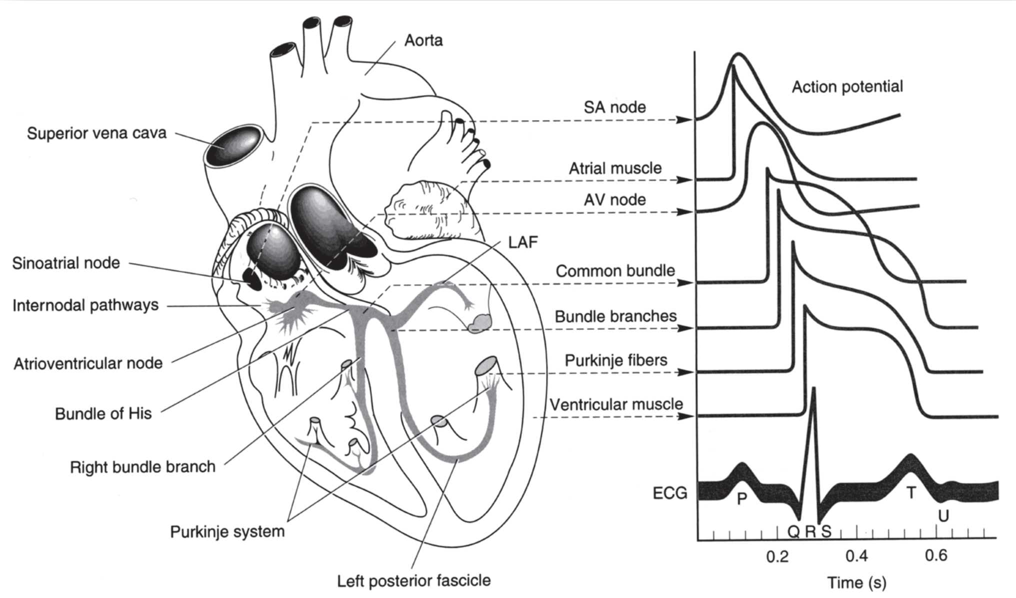

FIGURE 8-3. Conduction system of the heart. Typical transmembrane action potentials for the sinoatrial (SA) and atrioventricular (AV) nodes, other parts of the conduction system, and the atrial and ventricular muscles are shown along with the correlation to the extracellularly recorded electrical activity (i.e., the electrocardiogram [ECG]). The action potentials and ECG are plotted on the same time axis but with different zero points on the vertical scale. The PR interval is measured from the beginning of the P wave to the beginning of the QRS. (LAF, left anterior fascicle.) *Source:* Reproduced with permission from Kusumoto F. Cardiovascular disorders: heart disease. In: McPhee SJ, Lingappa VR, Ganong WF et al. Pathophysiology of disease: an introduction to clinical medicine. 3rd ed. New York: McGraw-Hill; 2000.

information on various aspects of heart chambers and coronary arteries.

Electrocardiographic Findings in Acute Coronary Syndrome

In patients with ACS, the ECG is an essential tool providing immediate and invaluable data for expeditious establishment of diagnosis, determination of prognosis, and consideration of management options. A 12-lead ECG should be obtained within 10 minutes of a patient's presentation to the emergency department if ACS is suspected. Careful reading of the ECG by an experienced clinician provides information on the presence of myocardial ischemia, injury, or infarction. The leads in which ECG changes consistent with ACS occur provide information on the occluded coronary artery most likely causing the ischemia or infarction (**Table 8-1**).

The classic ECG changes consistent with acute presentation of myocardial ischemia or infarction are (1) T-wave inversion, (2) ST-segment elevation, and (3) ST-segment depression (**Figure 8-4**). Q waves, defined by a width of >1 mm and a depth of >25% of the QRS complex height, are also indicative of MI and loss of electrically functioning cardiac tissue. Pathologic Q waves may appear within one to two hours, often 12 hours, and, occasionally, up to 24 hours of onset of symptoms, often within.[3] Among patients presenting with ST-segment elevation, most ultimately develop a Q-wave MI (QwMI); this is in contrast to the majority of patients presenting with NSTEMI who ultimately develop a non-Q wave MI (NQMI).[4]

ECG manifestations of non-ST elevation ACS are ST depression and T-wave inversion without ST segment elevation or pathologic Q waves. ECG criteria for the diagnosis of ST elevation MI (in the absence of left bundle branch block and left ventricular hypertrophy) include new ≥2 mm of ST segment elevation in the two contiguous leads for men ≥40 years, ≥2.5 mm in men <40 years, or ≥1.5 mm for women in leads V_2–V_3 and ≥1 mm in other leads. Criteria for ST depression include new ≥0.5 mm horizontal or down-sloping ST depression and T-wave inversion of ≥1 mm in two contiguous leads with prominent R-wave or R/S ratio >1.[1,5]

During an acute STEMI, the ECG evolves through three stages, any one of which may be present without any of the others:

TABLE 8-1. Localization of Left Ventricular Myocardial Infarction by Anatomical Relationships of Leads

ANATOMIC SITE	CORONARY ARTERY MOST LIKELY INVOLVED	ECG LEADS WITH ISCHEMIC CHANGES
Inferior wall	RCA	II, III, and aVF
Anterior wall	LAD	V_1 to V_4
Lateral wall	CX	I, aVL, V_5, and V_6
Posterior	RCA	V_1 – V_3

CX = circumflex branch of the left coronary artery; ECG = electrocardiogram; LAD = left anterior descending artery; RCA = right coronary artery.

- T-wave peaking with subsequent T-wave inversion—With the onset of infarction, the T waves become hyperacute or tall and narrow, commonly known as *peaking*. Shortly thereafter, usually within hours, the T waves invert. T wave changes are reflective of myocardial ischemia, but they are not indicative of an MI.
- ST-segment elevation signifies myocardial injury, likely reflecting a degree of cellular damage beyond that of mere ischemia; however, this is potentially reversible. This is a more reliable sign that is diagnostic of true infarction. Persistent ST segment elevation may indicate other cardiac injury such as ventricular aneurysm.
- Appearance of new Q-waves, which is indicative of irreversible myocardial cell death (diagnostic of an MI)

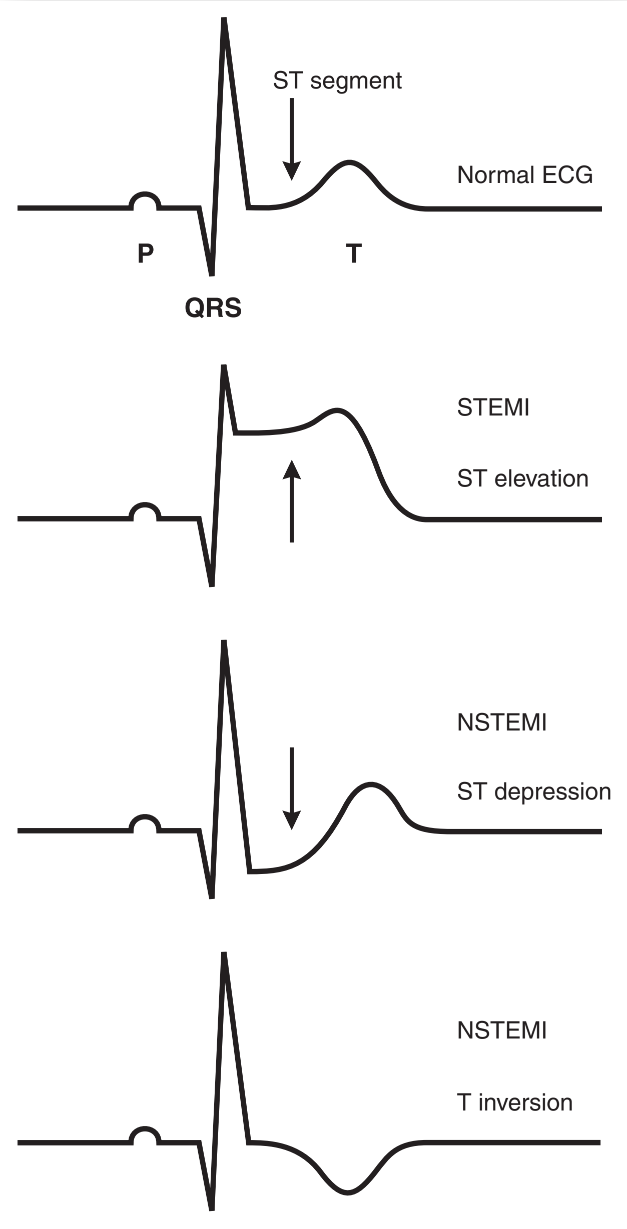

FIGURE 8-4. ECG changes consistent with STEMI and NSTEMI.

MINICASE 1

Acute Coronary Syndrome

Ethan W., a 68-year-old man with history of hypertension, dyslipidemia, and type 2 diabetes, presents to the emergency department complaining of substernal chest discomfort, which radiates to the left arm, shortness of breath, and palpitations for the past four hours. He appears in distress. His vital signs include BP 150/90 mm Hg, HR 130 beats/min, and RR 24 breaths/min. His jugular venous pressure (JVP) is normal, and his lungs are clear. Cardiac exam reveals tachycardia with no murmurs or rub appreciated. A benign abdominal exam, with no hepatojugular reflux and lower extremities, reveals no edema. Chest x-ray does not show any evidence of cardiomegaly or congestion. ECG reveals ST elevation in anterior leads. At presentation, troponin I is 9 ng/mL, and BNP is 300 pg/mL. An echocardiogram reveals normal left ventricular size with an estimated ejection fraction of 50% and anterior wall motion akinesis.

QUESTION: What is the most likely assessment of this patient's presentation?

DISCUSSION: This patient is considered at high risk for cardiac events given his history of diabetes, hypertension, and dyslipidemia. Based on the ECG findings, along with the symptoms and the elevated troponin level at presentation, he is experiencing an acute anterior ST-segment elevation myocardial infarction (STEMI). In addition, the wall motion abnormality noted on echocardiography is consistent with MI. He is not showing evidence of heart failure on exam, and chest x-ray reveals no evidence of congestion. Elevated BNP levels in acute coronary syndrome have been shown to be prognostic of a poor outcome, even in the absence of clinical evidence of heart failure.

In addition to aiding in the diagnosis of ACS, ECG findings provide prognostic information and are key parameters in monitoring for efficacy of therapy in the acute setting. **(Minicase 1.)**

LABORATORY TESTS USED IN THE EVALUATION OF ACUTE CORONARY SYNDROME

MI can be recognized by clinical presentation, electrocardiography, elevated biochemical markers of myocardial necrosis, and imaging.[1,6] Clinical presentation does not distinguish among UA, NSTEMI, and STEMI. The ECG differentiates between NSTEMI and STEMI. Unstable angina/non-ST-segment elevation MI (NSTEMI) is defined by ST-segment depression or prominent T-wave inversion and positive biomarkers of necrosis (e.g., troponin) in the absence of ST-segment elevation and in an appropriate clinical setting (chest discomfort or anginal equivalent). The distinction between UA and NSTEMI is ultimately made on the basis of the absence or presence, respectively, of biochemical cardiac markers in the blood. The release of detectable quantities of biochemical markers in the peripheral circulation indicates myocardial injury and is more consistent with MI than UA. Markers are detected in the peripheral circulation within a few hours after the initial insult in NSTEMI and STEMI.

In the era of reperfusion therapy, diagnosing ACS accurately and without delay is crucial for risk stratification and appropriate, life-saving treatment implementation. This section describes the laboratory tests used in the diagnosis of ACS. Special emphasis is placed on cardiac biomarkers. Other non-cardiac-specific tests are presented briefly.

Biochemical Cardiac Markers

Infarction of myocardial cells disrupts membrane integrity, leaking intracellular macromolecules into the peripheral circulation where they are detected. The criteria of an ideal biochemical marker for the diagnosis of ACS include the following[6]:

1. High specificity: Present in high concentrations in the myocardial tissues and absent from nonmyocardial tissue
2. High sensitivity: Detects minor injury to the myocardium
3. Release and clearance kinetics provide expedient and practical diagnosis
   a. Rapidly released into the blood after injury to facilitate early diagnosis
   b. Persists for sufficient time to provide convenient diagnostic time window
4. Measured level of the marker is in direct proportional relationship to the extent of myocardial injury.
5. Assay technique is commercially available and is easy to perform, inexpensive, and rapid.

Several biochemical cardiac markers are used in the diagnosis and evaluation of ACS. The cardiac-specific troponins have a number of attractive features and have gained acceptance as the biochemical markers of choice in the evaluation of patients with ACS.[1,4,5]

Cardiac-Specific Troponin I

Diagnostic level: ≥0.3 ng/mL (≥0.3 mcg/L) (assay dependent)

Cardiac-Specific Troponin T

Diagnostic level: ≥0.1 ng/mL (≥0.1 mcg/L) (assay dependent)

Troponin is a protein complex consisting of three subunits: troponin C (TnC), troponin I (TnI), and troponin T (TnT). The three subunits are located along thin filaments of myofibrils, and they regulate Ca^{+2}-mediated interaction of actin and myosin necessary for the contraction of cardiac muscles. Troponin C binds Ca^{+2}, TnI inhibits actomyosin ATPase, and TnT attaches to tropomyosin on the thin filaments. The TnC expressed by myocardial cells in cardiac and skeletal muscle is identical. In contrast, TnI and TnT expressed by cardiac cells are encoded by distinct genes different from those in skeletal muscle cells. Distinct amino acid sequences between the two isoforms allow for specific antibody development without cross-reactivity. Monoclonal antibody-based immunoassays have been developed to detect cardiac-specific TnI (cTnI) and cardiac-specific TnT (cTnT).

Cardiac-specific TnI and cTnT are highly specific and sensitive for MI.[7-9] Following myocardial injury, serum cTnI and cTnT begin to rise above the upper reference limit within 3–12 hours, peak in 24 hours (cTnI) or 12 hours to 2 days (cTnT), and return to normal in 5–10 days (cTnI) or 5–14 days (cTnT) (**Table 8-2**). The initial rise of troponin is due to the release of cytoplasmic troponin whereas the later sustained rise is due to the release of complexed troponin from disintegrating myofilaments (**Figure 8-5**).[10] Levels typically increase more than 20 times above the reference limit. The prolonged time course of elevation of cTnI and cTnT is useful for the late diagnosis of MI.

Serial troponin levels should be obtained at presentation and three to six hours after onset of symptoms. A level of troponin T or I that exceeds the decision level on at least one occasion during the first 24 hours after an index clinical ischemic event indicates MI. A pattern that shows rising and falling troponin levels is required for the diagnosis of ACS. This is especially helpful in differentiating troponin elevation due to MI from that due to chronic conditions. Additional troponin levels should be obtained beyond six hours if the clinical index of suspicion for ACS is high.[4]

Several analytical factors should be considered with troponin assays. The first-generation troponin assay T ELISA was limited by a lack of specificity to cardiac troponin and a long turnaround time (90 minutes at 20 °C and 45 minutes at 37 °C). Second-generation troponin assay T ELISA was improved by changing the antibody detected to the cardiac-specific antibody M11.7, resulting in enhanced specificity.[11] The third-generation assay (Elecsys, Roche Diagnostics) uses recombinant human cardiac troponin T as standard material enabling reproducibility and standardization of cTnT assays with a normal cutoff concentration of 0.1 mcg/L and a turnaround time of 9–12 minutes.[12]

In contrast to cTnT, cTnI assays lack standardization among multiple commercially available assays developed by different manufacturers. A number of factors complicate the standardization of the assays including (1) cTnI released from disintegrating myocytes may be free cTnI, complexed with cTnC, or a combination of the two forms as well as free-cTnI degradation products; (2) the different forms undergo oxidation, phosphorylation, and proteolysis after release from cells; and (3) the matrices used and the commutability from an artificial matrix to a physiologic one vary among assays.[13] Despite these differences, most commercial immunoassays measure cTnI. Contemporary cTnI assays have an analytical sensitivity almost 100-fold higher than that of the first available commercial assays. Different specificities of the antibodies used for detecting free and complexed cTnI may lead to variations in the cutoff concentrations or abnormal levels of cTnI in the available immunoassays. Considerable variation (up to 20-fold) in cTnI levels may be observed when measured by different methods causing ambiguity in clinical interpretation.[14-17]

Commercially available troponin assays vary widely in lower detection limits, upper reference limits, diagnostic cut points, and assay imprecision (coefficient of variation). The upper reference limit is established by each laboratory and is set at the 99th percentile of normal population. An increased value for cardiac troponin is defined as a measurement exceeding the 99th percentile of a reference control group. Acceptable imprecision at the 99th percentile for each assay is defined as ≤10.[18] However, not all commercially available assays can achieve this precision level. Thus, when interpreting results, clinicians should employ the upper reference limit and MI diagnostic cutoff values for the particular assay used in each institution's laboratory. **Table 8-3** provides an example of one institution's interpretive data for cTnI.

Cardiac troponins have been endorsed internationally as the standard biomarkers for the detection of myocardial injury, diagnosis of MI, and risk stratification in patients with suspected ACS.[1,4,5,19] Significant prognostic information may be inferred from troponin levels. In a study of patients presenting to the emergency department with chest pain, negative qualitative bedside testing of cTnI and cTnT was associated with low risk for death or MI within 30 days (event rates of 0.3 and 1.1, respectively).[20] Other large clinical trials have documented that elevated troponin levels are strong, independent predictors of mortality and serious adverse outcome 30–42 days after ACS.[21-25] Troponin levels should always be used in conjunction with other clinical findings. In one study, in-hospital mortality was as high as 12.7% in a troponin-negative subgroup of patients with ACS.[26]

TABLE 8-2. Biochemical Markers Used in the Diagnosis of ACS

MARKER	MOLECULAR WEIGHT (daltons)	RANGE OF TIME TO INITIAL ELEVATIONS (hr)	MEAN TIME TO PEAK ELEVATIONS (nonthrombolysis)	TIME TO RETURN TO NORMAL RANGE
cTnI	23,500	3–12 hr	24 hr	5–10 days
cTnT	33,000	3–12 hr	12 hr–2 days	5–14 days
CK-MB	86,000	3–12 hr	24 hr	2–3 days
Myoglobin	17,800	1–4 hr	6–7 hr	24 hr

CK-MB = creatinine kinase isoenzyme MB; cTnI = cardiac-specific troponin I; cTnT = cardiac-specific troponin T.

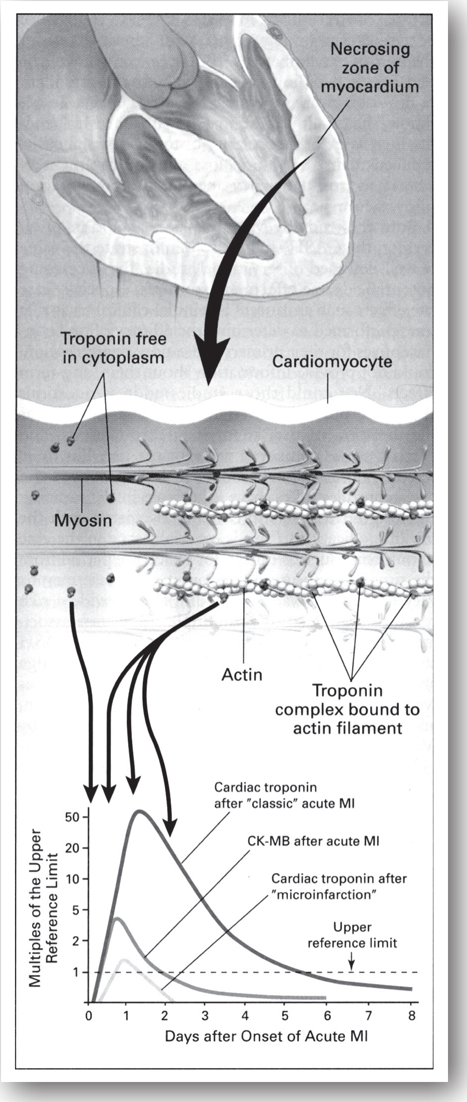
Necrosing zone of myocardium
Troponin free in cytoplasm
Cardiomyocyte
Myosin
Actin
Troponin complex bound to actin filament
Cardiac troponin after "classic" acute MI
CK-MB after acute MI
Cardiac troponin after "microinfarction"
Upper reference limit
Multiples of the Upper Reference Limit
50
20
10
5
2
1
0
1
2
3
4
5
6
7
8
Days after Onset of Acute MI

FIGURE 8-5. Release of cardiac troponins in MI. The zone of necrosing myocardium is shown at the top of the figure, followed in the middle portion of the figure by a diagram of a cardiomyocyte that is in the process of releasing biomarkers. Most troponin exists as a tripartite complex of C, I, and T components that are bound to actin filaments, although a small amount of troponin is free in the cytoplasm. After disruption of the sarcolemmal membrane of the cardiomyocyte, the cytoplasmic pool of troponin is released first (left-most arrow in bottom portion of figure), followed by a more protracted release from the disintegrating myofilaments that may continue for several days (three-headed arrow). Cardiac troponin levels rise to about 20–50 times the upper reference limit (the 99th percentile of values in a reference control group) in patients who have a classic AMI and sustain sufficient myocardial necrosis to result in abnormally elevated levels of the MB fraction of creatine kinase (CK-MB). Clinicians can now diagnose episodes of microinfarction by sensitive assays that detect cardiac troponin elevations above the upper reference limit, even though CK-MB levels may be still in the normal reference range. *Source:* Reprinted with permission from Antman EM. Decision-making with cardiac troponin tests. *N Engl J Med.* 2002; 346:2079–82. Copyright ©2002, Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society.

TABLE 8-3. An Example of Interpretive Data for Troponin I

REFERENCE RANGE: 0.00–0.02 ng/mL (0.00–0.02 mcg/L)

Comments:
The 99th percentile for healthy adults is ≤0.02 ng/mL or ≤0.02 mcg/L.
Probable MI is indicated at ≥0.3 ng/mL (≥0.3 mcg/L).

Hours postadmit	0–6	6–12	12–24
% sensitivity	60	79	92
% specificity	95	95	95

MI = myocardial infarction.

High-sensitivity troponin I (hsTnI) and troponin T (hsTnT) assays have been developed to increase the clinical sensitivity for detection of myocardial injury. High-sensitivity troponin assays detect concentrations of the same proteins that conventional sensitivity assays are aimed at detecting but in much lower concentrations. These assays have substantially lower limits of detection (in the picogram per milliliter range versus the current assays in the nanogram per milliliter range) as well as improved assay precision. To be classified as high-sensitivity assays, concentrations below the 99th percentile should be detectable above the assay's limit of detection for >50% of healthy individuals in the population of interest. High-sensitivity assays, by expert consensus, should have a coefficient of variance (CV) of <10% at the 99th percentile value in the population of interest.[18,27-29] Studies suggest that high-sensitivity troponins provide enhanced diagnostic and prognostic accuracy. In one study, hsTnT was superior to TnT but equivalent to third-generation TnI for the diagnosis of MI, and hsTnT was the most likely assay to be elevated at baseline. The study also showed that change in troponin levels (δ-troponin) increase specificity but reduce sensitivity for the detection of acute MI.[30] Another study comparing hsTnI (Architect STAT hsTnI assay, Abbott Diagnostics) and cTnI (Architect STAT cTnI assay, Abbott Diagnostics) revealed that measurement at three hours after admission may help rule out MI. Troponin measured using either assay was superior to other biomarkers (including CK and CK-MB) in ruling in or ruling out MI. The sensitivity and negative predictive value of the hsTnI assay were higher than the cTnI assay at admission (82.3% and 94.7% versus 79.4% and 94%, respectively); however, the negative predictive value of both assays was 99.4% at three hours. For patients with detectable troponin on admission (using the 99th percentile diagnostic cutoff value) and a 250% increase in troponin at three hours, the probability of MI was 95.8%.[31] Several high-sensitivity troponin assays are available in Europe: Roche Elecsys hs cardiac troponin T (hs-cTnT), Elecsys hs-cTnT STAT, Abbott ARCHITECT STAT hs cardiac troponin I (hs-cTnI), and Trinity Meritas hs-cTnI assay. The use of high-sensitivity troponins in patients with suspected ACS is currently in clinical use in Europe.[19] A recent study comparing hs-cTnI (Abbott) and hs-cTnT (Roche) in clinical use showed that both provided high diagnostic and prognostic accuracy.[32] The clinical use of δ-troponin in addition to troponin levels may further enhance diagnostic accuracy. Studies have evaluated δ-troponin using high-sensitivity troponin assays in patients presenting with MI, with promising findings.[30,33,34] High-sensitivity troponin assays are not commercially available in the United States. Potential challenges to consider before incorporating high-sensitivity troponin assays into clinical practice include false-positive interpretation, biologic variability, and lack of assay standardization.[35]

A number of cardiac and noncardiac conditions have been reported to cause detectable serum levels of troponins in the absence of ACS (**Table 8-4**).[36-38] The use of cardiac troponin levels in patients with renal dysfunction has been controversial.[39-42] Most data indicate that cTnI is highly specific, but it should be considered as a useful but an imperfect marker in patients with renal insufficiency and end-stage renal disease.[39] In patients with end-stage renal disease and no evidence of acute myocardial necrosis, <10% will have elevated cTnI and a significantly higher number will have elevated cTnT. This is thought to be due to silent subendocardial ischemia, decreased clearance, or other metabolic abnormalities. Regardless of cause, it is well established that these patients with elevated cardiac troponin levels have a higher risk of mortality even in the absence of symptoms.[41] Dynamic changes in troponin level of >20% within nine hours (with a minimum of one value exceeding the 99th percentile) is recommended for an MI diagnosis among patients with end-stage renal disease and suspected ACS.[43] Troponin levels may be useful for U.S. Food and Drug Administration (FDA)-approved troponin testing for prognosis in patients with chronic kidney disease and patients undergoing chemotherapy who have drug-induced cardiac injury. Troponin has been used to risk stratify patients undergoing high dose chemotherapy with evidence of worst outcomes noted with higher levels. Some studies have advocated for use of cardioprotective drug regimens in patients with elevated troponin levels to reduce risks.[44-46]

TABLE 8-4. Causes of Detectable Serum Levels of Troponins in the Absence of Acute Coronary Syndrome

- Aortic dissection
- Bradycardia or tachycardia
- Burns affecting >30% of body surface area
- Cardiac contusion or trauma (cardiac surgery, ablation, pacing, implantable cardioverter-defibrillator shocks, cardioversion, endomyocardial biopsy)
- Cardiomyopathy
- Cardiotoxicity (doxorubicin, fluorouracil, trastuzumab)
- Cardiopulmonary resuscitation
- Coronary angioplasty or vasospasm
- Critical illness (respiratory failure, sepsis)
- Heart failure (chronic and acute decompensation)
- Heart transplant rejection
- Infiltrative disorders with cardiac involvement (amyloidosis, sarcoidosis)
- Left ventricular hypertrophy
- Myocarditis or pericarditis
- Neurological diseases, acute (cerebrovascular accident, subarachnoid hemorrhage)
- Pulmonary embolism or severe pulmonary hypertension
- Rhabdomyolysis with cardiac injury
- Renal failure and hemodialysis

Source: Adapted from references 36–38.

In patients undergoing PCI, clinical evidence of MI and a troponin elevation greater than five times the upper limit of normal defines a PCI-related MI, with a higher risk of death, cardiac complications, and need for repeat revascularization.[47] One meta-analysis showed that troponin elevation after PCI was associated with increased mortality, and that mortality or nonfatal MI was more likely to occur in patients with a post-PCI troponin elevation.[48]

Creatine Kinase

Normal range: males, 55–170 IU/L (0.92–2.84 µkat/L); females, 30–135 IU/L (0.5–2.25 µkat/L)

Creatine kinase (CK) is an enzyme that stimulates the transfer of high-energy phosphate groups, and it is found in skeletal muscle, myocardium, and the brain. Circulating serum CK is directly related to the individual's muscle mass.

Serum CK concentrations rise sharply four to eight hours after the onset of chest pain associated with MI, peak in 24 hours, and return to normal in three to four days. Maximum concentrations of CK may reach five to seven times the normal values. Serial CK measurements following MI provide excellent sensitivity (98%) but poor specificity (67%). Other causes for increased serum CK concentrations include any musculoskeletal injury or diseases, intramuscular injections, and a number of medications (**Table 8-5**). Given the availability and characteristics of cardiac troponins, CK and CK-MB measurements are no longer useful for the diagnosis of ACS.

Creatine Kinase Isoenzymes

Normal range: CK-MB ≤6 ng/mL (≤6 mcg/L)

The enzyme CK is a dimer of two B monomers (CK-BB), two M monomers (CK-MM), or a hybrid of the two (CK-MB). The three isoenzymes are found in different sources: CK-BB is found in the brain, lungs, and intestinal tract; CK-MM is found primarily in skeletal and cardiac muscle; and CK-MB is found predominantly in the myocardium but also in skeletal muscle. Fractionation of total CK into three isoenzymes increases the diagnostic specificity of the test for MI.

The CK-MB isoenzyme is most specific for myocardial tissue and has been used for the diagnosis of ACS. Serum CK-MB concentrations begin to rise 3–12 hours after the onset of symptoms, peak in 24 hours, and return to baseline in 2–3 days. Other causes for elevated CK-MB levels include trauma, skeletal muscle injury, or surgical procedures involving the small intestine, tongue, diaphragm, uterus, or prostate.

Electrophoretic separation followed by fluorometric analysis of isoenzyme bands is used to determine the actual CK-MB concentration in IU per liter (IU/L). The cutoff for the upper limit of normal when the actual CK-MB concentration is determined ranges between is 5–25 IU/L. Alternatively, CK-MB as a percentage of the total CK concentration is determined by dividing the CK-MB concentration by the total CK

TABLE 8-5. Causes of Elevated CK Levels

SKELETAL MUSCLE CAUSES	CARDIAC CAUSES
Dermatomyositis	Myocarditis
Polymyositis	Pericarditis
Muscular dystrophy	MI
Myxedema	
Malignant hyperpyrexia	
Vigorous exercise	
Malignant hyperthermia syndrome	
Rhabdomyolysis	
Delirium tremens	
Seizures	
Trauma	

MEDICATIONS	OTHER CAUSES
HMG-CoA reductase inhibitors	Hypothyroidism
Amphotericin B	Renal failure
Clofibrate	Cerebrovascular accident
Ethanol (binge drinking)	Pulmonary embolism
Lithium	Severe hypokalemia
Halothane	
Succinylcholine	
Barbiturate poisoning	
Large doses of aminocaproic acid	
IM injections	

CK = creatine kinase; HMG-CoA = 3-hydroxy-3-methylglutaryl-coenzyme A; IM = intramuscular; MI = myocardial infarction.

concentration. The cutoff for the upper limit of normal for this value is usually 3–6%. The diagnosis of MI is strongly suggested when CK-MB as a percentage of total CK is >5% or when the actual CK-MB concentration is >10 IU/L.

When interpreting serum CK-MB levels, the timing of blood specimen collections in relation to the onset of symptoms must be assessed. Lack of absolute cardiac specificity (skeletal muscle, healthy persons) limits CK-MB interpretation. Other causes for elevated CK-MB levels are listed in **Table 8-6** and are usually not associated with the typical rise and fall in serum levels as seen in ACS. To differentiate between cardiac and noncardiac sources of CK-MB elevation, the relative index (RI), or the ratio of CK-MB to total CK concentrations, can be used and is calculated using the following equation:

$$\mathrm{RI} = \frac{\text{CK-MB}}{\text{Total CK}} \times 100$$

This index is usually calculated only when both total CK and CK-MB are elevated above the normal value. It is useful in differentiating MB released from cardiac versus skeletal muscle. An index >5 indicates significant myocardial injury. CK-MB is no longer recommended in clinical practice for the diagnosis of ACS; however, it may be used to estimate size of infarct.[4]

TABLE 8-6. Causes of Elevated CK-MB Levels in the Absence of Acute Coronary Syndromse

Athletic activity (e.g., marathons)
Cardiac surgery
Hyperthermia/Hypothermia with cardiac involvement
Hypothyroidism
Malignancy
Muscular dystrophy
Myocardial puncture/trauma
Myocarditis
Myositis
Pericarditis
Pulmonary embolism
Renal failure
Rhabdomyolysis
Stroke and subarachnoid hemorrhage
Surgery (gastrointestinal, prostate)

CK-MB Isoforms

Once *CK-MB*, also known as the *CK-MB*$_2$ isoform while in the myocardial tissue, is released into the circulation following an MI, it undergoes metabolism by lysine carboxypeptidase producing the more negatively charged isoform CK-MB$_1$. In healthy individuals, the two isoforms are in equilibrium and the normal levels are 0.5–1 IU/L for each isoform. One study showed that elevated CK-MB$_2$ levels of >1 IU/L and an increased ratio of CK-MB$_2$ to CK-MB$_1$ of 1.5 has a sensitivity of MI diagnosis in the emergency department of 59% when measured at two to four hours and 92% at four to six hours postonset of symptoms.[49] Another study showed that CK-MB isoforms were most sensitive and specific (91% and 89%, respectively) when measured six hours after onset of chest pain in patients with MI presenting to the emergency department.[50] Similar to CK-MB, the isoforms lack cardiac specificity and are of no use in the clinical assessment of patients with ACS.

Myoglobin

Myoglobin is a low-molecular-weight heme protein found in cardiac and skeletal muscle. Serum levels are detected within one to four hours and peak six to seven hours after the onset of symptoms. Myoglobin is cleared rapidly by renal glomerular filtration, and levels return to the reference value 24 hours following MI. The fast rise and fall of myoglobin levels made it an appropriate marker for the early diagnosis of MI and detection a reinfarction if occurring 24 hours after the initial insult. With the advent of cardiac troponins as the standard biomarker for the diagnosis and prognosis of patients presenting with ACS, myoglobin measurement is rendered obsolete in current practice.

Other Biochemical Markers

Two other biochemical markers have been shown useful in patients with ACS: *B-type natriuretic peptide* (BNP) and *c-reactive protein* (CRP). BNP is a neurohormone released by ventricular myocardium in response to volume overload. It is used for the evaluation of patients with heart failure. However, it has been shown to be a strong predictor of short-term and long-term mortality in patients with ACS. A detailed discussion of BNP is presented later in the chapter. CRP is a nonspecific acute-phase reactant that is released in the presence of inflammatory processes caused by various etiologies (e.g., infections, malignancy, trauma, rheumatoid arthritis, and other inflammatory processes). It is synthesized in the liver and is normally present in trace amounts in the peripheral circulation. In the presence of inflammation, CRP production is stimulated by systemic cytokines. Because inflammation plays a role in the pathophysiologic processes leading to atherosclerosis and plaque rupture, serum and plasma levels of CRP are elevated in patients with CAD and ACS. Several studies have reported CRP to be a strong predictor of mortality in patients presenting with ACS.[51-55] In addition, the risks of recurrent MI and death in patients with ACS receiving aggressive statin therapy were decreased significantly more in patients with low CRP levels than those with higher CRP levels regardless of the low-density lipoprotein cholesterol level.[56]

Several CRP qualitative and quantitative assays are commercially available. The diagnostic level cutoff is assay dependent. CRP assays lack the sensitivity necessary to measure low levels of the protein that might be found in healthy individuals for prognostic purposes; they are intended for use in the acute setting for the evaluation of the presence of infection, tissue injury, and inflammatory disorders. In addition, as a nonspecific marker of inflammation, the test is used to indicate the presence of inflammation, but it is not useful in delineating the cause of the inflammation. Measuring CRP for the diagnosis of CAD or ACS or cardiovascular risk stratification is not recommended for routine use in clinical practice.

High-sensitivity C-reactive protein (hsCRP) detects low levels (0.2–0.4 mg/L) with sufficient precision needed for accurate atherosclerotic cardiovascular risk assessment in otherwise healthy individuals. hsCRP levels have been shown to be a strong independent predictor of cardiovascular and peripheral vascular disease risk and of recurrent cardiac events in patients with history of CAD or ACS. Patients with hsCRP levels ≤0.9 mg/L are stratified to have low cardiovascular risk. Those having levels between 1 and 3 mg/L and 3.1 and 10 mg/L are stratified to have average and high cardiovascular risk, respectively.[57] The evaluation of hsCRP should be performed in conjunction with the standard cholesterol evaluation for cardiovascular disease risk stratification. The average of two measurements of hsCRP performed two weeks apart should be used. Patients with active infections or systemic inflammatory processes have increased hsCRP levels. To enhance the accuracy of cardiovascular risk assessment in these patients, hsCRP measurement should be postponed until the acute phase of the active infection or systemic inflammatory process has subsided and the patients are metabolically stable.[58]

In 2005, the FDA delineated another type of designation to a high-sensitivity CRP assay, the cardiac CRP (cCRP).[59] The guidance provided manufacturers with recommendations for development of CRP assays, specifically that cCRP is exclusively indicated for the assessment and stratification of individuals at risk for cardiovascular disease, whereas hsCRP is indicated for the evaluation of other conditions thought to be associated with inflammation, in otherwise healthy individuals. The diagnostic cutoff for cCRP is the same as that of hsCRP. The difference between the hsCRP and cCRP assays is not the analyte used, but rather the additional performance validation required for the expanded indication for use in the evaluation of CAD and ACS. The need to differentiate between the two types of CRP assays has been debated.[60,61]

Even though it has no role in the diagnosis of ACS, when used in conjunction with standard clinical laboratory evaluation of ACS, cCRP may be useful as an independent marker of prognosis for recurrent events, in patients with stable coronary disease, or ACS.[62-65] Several studies have shown higher CRP levels to correlate with higher occurrence of atrial fibrillation in patients undergoing cardiac surgery.[66-68] The 2014 guideline for the management of patients with atrial fibrillation suggest that colchicine, as an anti-inflammatory agent that decreases circulating CRP levels, may be considered postoperatively to reduce atrial fibrillation after cardiac surgery.[69-73] Additional studies are needed to fully define the correlation between CRP levels and the occurrence of atrial fibrillation and colchicine's efficacy and appropriate use in these patients.

Other biochemical markers such as serum amyloid A and interleukin-6, which are acute phase inflammation markers, have been shown to predict an increased risk of adverse outcomes of ACS patients.[74,75] Fibrinopeptide and fibrinogen, two markers of coagulation cascade activity, also appear to be associated with an increased risk and a poor clinical outcome in patients with UA.[76,77] Myeloperoxidase, a hemoprotein with microbicidal activity has been shown to have proatherogenic properties, leading to CAD and ACS. Several studies have revealed that elevated serum and plasma levels of myeloperoxidase are independent predictors of outcomes in patients presenting with ACS.[78-80] In addition, microalbuminuria has been shown in a limited number of studies to be an independent predictor of CAD and a strong prognostic marker in patients with AMI.[81-84]

Miscellaneous Laboratory Tests

A number of noncardiac specific laboratory abnormalities may be manifested in patients with MI. These include nonspecific elevation of serum glucose, white blood cells, and erythrocyte sedimentation rate as well as alterations in lipid profile findings. Total cholesterol and low-density lipoprotein may be decreased when measured 48–72 hours post-MI and may persist for six to eight weeks afterward. Recognition of these abnormalities as secondary to MI precludes misinterpretation or misdiagnosis of other disorders.

Recommendations for Measurement of Biochemical Markers

Cardiac troponins (cTnI or cTnT) are the preferred cardiac biochemical markers for the evaluation of patients presenting with suspected ACS. Cardiac troponins should be measured at presentation and repeated after three to six hours. Time of presentation from onset of symptoms should be taken into account. Follow-up troponin levels should be obtained beyond six hours in patients who have ECG changes or are considered intermediate or high risk for ACS. A single troponin measurement at day 3 or 4 may be obtained to assess infarct size. Measuring BNP may be reasonable for additional prognostic information.

LABORATORY TESTS USED IN THE EVALUATION OF HEART FAILURE

Natriuretic Peptides

Natriuretic peptides are naturally secreted hormones that are released by various cells in response to increased volume or pressure. A number of natriuretic peptides have been identified, including atrial natriuretic peptide (ANP), BNP, C-type natriuretic peptide (CNP), dendroaspis natriuretic peptide (DNP), and urodilatin. The latter two were recently isolated from the venom of *Dendroaspis angusticeps* (green mamba snake) and human urine, respectively. However, their clinical utilization in patients with cardiovascular diseases is not well defined as of this writing. CNP, a structurally related peptide to ANP and BNP, is secreted by the heart and vascular endothelium and exerts vasodilating effects.[85] Limited evidence suggests that plasma CNP levels are increased in patients with heart failure, but further investigation of the clinical utilization of CNP levels and their interpretation in the diagnosis and assessment of heart failure is warranted.[86]

ANP and BNP are known cardiac-specific peptides. The two are structurally similar and exert potent diuretic, natriuretic, and vascular smooth muscle-relaxing effects. A 28-amino acid (aa) peptide, ANP is primarily secreted by the atrial myocytes in response to increased atrial wall tension. A 32-aa peptide, BNP was first identified in porcine brain extracts (hence the name *brain natriuretic peptide*).[87] Subsequently, it was found in much higher concentrations in cardiac ventricles and is primarily secreted by the left ventricular myocytes in response to volume overload and increased ventricular wall tension.

The precursor for BNP is PreproBNP, a 134-aa peptide that is enzymatically cleaved into proBNP, a 108-aa peptide. The latter is then further cleaved into the biologically active C-terminal 32-aa BNP and the biologically inactive amino-terminal portion of the prohormone, N-terminal-proBNP (NT-proBNP). Plasma levels of both BNP and NT-proBNP are elevated in response to increased volume and ventricular myocyte stretch in patients with heart failure. Once released into the peripheral circulation, BNP is cleared by enzymatic degradation via endopeptidase and natriuretic peptide receptor-mediated endocytosis, whereas NT-proBNP is cleared renally. The elimination half-life of BNP is significantly shorter than that of NT-proBNP (20 minutes versus 120 minutes, respectively).

The quantitative measurements of BNP and NT-proBNP levels are indicated for the evaluation of patients suspected of having heart failure, assessment of the severity of heart failure, and risk stratification of patients with heart failure and ACS.[88] In conjunction with standard clinical assessment, BNP and NT-proBNP levels at the approved cutoff points are highly sensitive and specific for the diagnosis of acute heart failure and correlate well with the severity of heart failure symptoms as evaluated by the New York Heart Association (NYHA) Classification.[89,90] In addition, BNP and NT-proBNP are strong independent markers of clinical outcomes in patients with heart failure, IHD, and ACS even in the absence of previous history of congestive heart failure (CHF) or objective evidence of left ventricular dysfunction during hospitalization.[91-97]

The value of serial BNP and NT-proBNP measurements to guide optimal heart failure therapy has been investigated. Several randomized trials of chronic heart failure patients have compared standard heart failure therapy plus BNP or NT-proBNP-guided therapy to standard heart failure treatment alone.[98-103] A meta-analysis of these trials confirmed the findings that BNP-guided heart failure therapy reduces all-cause mortality in patients with chronic heart failure, compared with usual clinical care in patients younger than 75 years, but not in those older than 75 years of age. Mortality reduction might be attributable to the higher percentage of patients achieving target doses of angiotensin-converting enzyme inhibitors and β-blockers—classes of agents shown to delay or halt progression of cardiac dysfunction and improve mortality in patients with heart failure.[104] A >30% reduction in BNP levels in response to heart failure treatment indicates a good prognosis.[105] BNP levels cannot be used in the assessment of patients receiving therapy with the synthetic BNP, Natrecor (nesiritide, Scios). Natrecor is a natriuretic peptide indicated for the treatment of patients with acutely decompensated heart failure who have dyspnea at rest or with minimal activity. It is a recombinant form of human BNP that is detected by the blood assays used to monitor the level of natural BNP. As such, BNP measurement, by itself, does not differentiate between heart failure and the drug level. Assays measuring NT-pro-BNP are not affected by the presence of synthetic BNP and thus may be used in this scenario.

Several factors impact the BNP and NT-proBNP levels, including gender, age, renal function, and obesity. Plasma BNP and NT-proBNP levels in normal volunteers are higher in women and increase with age. In addition, renal insufficiency at an estimated glomerular filtration rate (GFR) below 60 mL/min/1.73 m^2 may impact the interpretation of the measured natriuretic peptides. Significant correlation between NT-proBNP level and GFRs has been shown, more so than that between BNP level and GFRs. This is because renal clearance is the primary route of elimination of NT-proBNP, and the measured levels of the biomarker are elevated in patients with mild renal insufficiency. Yet, evaluation of patients with GFRs as low as 14.8 mL/min/1.73 m^2 revealed that the test continues to be valuable for the evaluation of the dyspneic patient irrespective of renal function.[106] Higher diagnostic cutoffs for different GFR ranges may be necessary for optimal interpretation in patients with renal insufficiency.

MINICASE 2

Heart Failure

Ruth G. is a 76-year-old woman with a history of poorly controlled hypertension and CAD who presents to the emergency department with two weeks of progressive dyspnea on exertion and now shortness of breath at rest. She reports sleeping in a recliner for the last three nights in order to breathe more comfortably. She denies any chest discomfort and admits to smoking and medication nonadherence.

On examination, Ruth G. is unable to complete full sentences secondary to breathing difficulty. Her vital signs include BP 190/105 mm Hg, HR 100 beats/min, and RR 30 breaths/min. O_2 saturation is 86% on room air. Physical exam reveals elevated JVP at 18 cm H_2O. Lung exam reveals bibasilar dullness to percussion with diffuse crackles. Cardiac exam reveals a regular tachycardic rate; S1, S2, S3 with 2/6 holosystolic murmur at apex and laterally displaced point of maximal intensity. She has a positive hepatojugular reflux and 2+ pitting edema in the lower extremities, bilaterally. Chest x-rays reveal an enlarged cardiac silhouette with moderate bilateral effusions and cephalization of vasculature. Blood work is significant for sodium 132 mmol/L, potassium 3.7 mmol/L, BUN 30 mg/dL, creatinine 1.5 mg/dL with an estimated GFR 46 mL/min/1.73 m^2, troponin I level of 0.06 ng/mL (remained at same level with repeat measurements), and BNP level of 2156 pg/mL. Echocardiogram reveals a dilated left ventricle with global hypokinesis and moderately depressed systolic function with an estimated ejection fraction of 38%.

QUESTION: How should this patient's findings and laboratory values be interpreted?

DISCUSSION: This patient has multiple risk factors for heart failure including history of CAD and poorly controlled hypertension. Her clinical presentation is compatible with acute decompensated heart failure with evidence of volume overload on physical exam (elevated JVP, positive hepatojugular reflux, 2+ lower extremity pitting edema). Her chest x-ray confirms findings of heart failure. Her BNP level is also significantly elevated and is indicative of heart failure. The low troponin level that did not rise is likely due to a silent subendocardial ischemia given her poorly controlled hypertension and heart failure in the setting of a decreased creatinine clearance. The clinical presentation, BNP level, and LVEF of 38% measured by echocardiography—the findings are all consistent with a diagnosis of heart failure with reduced ejection fraction (HF*r*EF).

Plasma levels of BNP and NT-proBNP are reduced in obese patients, limiting the clinical interpretation of the tests in these patients. An inverse relationship between the levels of these markers and body mass index (BMI) is observed.[107,108] The exact mechanism for this is not known, but a BMI-related defect in natriuretic peptide secretion has been suggested.[109] In one study, NT-proBNP levels were found to be lower in obese patients presenting with dyspnea (with or without acute heart failure), but the test seemed to retain its diagnostic and prognostic capacity across all BMI categories.[110] Similarly in another study, in patients with advanced systolic heart failure, the test predicted worse symptoms, impaired hemodynamics, and higher mortality at all levels of BMI. Although BNP levels were relatively lower in overweight and obese patients, optimal BNP cutoff levels for prediction of death or urgent transplant in lean, overweight, and obese patients were reported to be 590 pg/mL, 471 pg/mL, and 342 pg/mL, respectively.[111] To increase the specificity of BNP levels for heart failure in obese and lean patients, a diagnostic cutoff level of ≥54 pg/mL for severely obese patients and a cutoff level of ≥170 pg/mL in lean patients have been suggested.[112]

Despite the fact that BNP and NT-proBNP have no role in the diagnosis of ACS, they are powerful prognostic markers and predictors of mortality in these patients.[105,113-116] The use of BNP levels in the assessment of cardiotoxicity associated with anthracycline chemotherapy has also been studied.[117-120] Several studies have shown an improvement in early detection of chemotherapy related cardiotoxicity when biomarkers such as BNP and hsTNI were utilized in addition to serial evaluation of left ventricular ejection fraction This could potentially translate to earlier intervention and improved outcome.[121,122] **(Minicase 2.)**

B-Type Natriuretic Peptide

Diagnostic cutoff: 100 pg/mL (100 ng/L)

The clinical diagnostic cutoff level for heart failure is a BNP level of >100 pg/mL. In addition to standard clinical evaluation, a BNP level of >100 pg/mL is associated with sensitivity and specificity of 90% for heart failure in a patient presenting with shortness of breath.[123] The test has a high negative predictive value in ruling out heart failure as a primary cause for the presentation. A BNP level of 100–500 pg/mL is suggestive, whereas a level >500 pg/mL is indicative of heart failure as the likely etiology of acute dyspnea (**Table 8-7**).[105]

A study investigating the prognostic value of BNP levels in patients with heart failure showed that the risk ratio of all-cause mortality and first morbid event (defined as death, sudden death with resuscitation, hospitalization for heart failure, or intravenous inotropic or vasodilator therapy for at least four hours) for patients with baseline BNP above the median level of 97 pg/mL was significantly higher than for patients with values below the median. Furthermore, the study revealed a significant quartile-dependent increase in mortality and first morbid event (baseline values for BNP in quartiles were <41, 41 to <97, 97 to <238, and ≥238 pg/mL). Patients with the greatest percent decrease in BNP from baseline to 4 and 12 months follow-up periods had the lowest morbidity and mortality, whereas patients with greatest percent increase in BNP had the highest morbidity and mortality.[124] Another study showed that admission BNP and cardiac troponin levels are significant, independent predictors

TABLE 8-7. Interpretation of BNP and NT-proBNP Levels in Patients with Acute Dyspnea

	HEART FAILURE UNLIKELY	GREY ZONE	HEART FAILURE LIKELY	
BNP[a]	<100 pg/mL	100–500 pg/mL	>500 pg/mL	
NT-pro-BNP[b]	<300 pg/mL		<50 yr	>450 pg/mL
			50–75 yr	>900 pg/mL
			>75 yr	>1800 pg/mL

[a] In patients with estimated glomerular filtration rate <60 mL/min/1.73 m^2 and body mass index. <35 kg/m^2, different decision limits must be used.
[b] In patients with estimated glomerular filtration rate <60 mL/min/1.73 m^2, different decision limits must be used.
Source: Adapted from reference 97.

of in-hospital mortality in acutely decompensated heart failure. Patients with BNP levels ≥840 pg/mL and increased troponin levels were at particularly high risk for mortality.[125]

Several assays using various technologies are available in the United States for the quantitative measurement of BNP: (1) AxSYM (Abbott Laboratories), which utilizes microparticle enzyme immunoassay; (2) ADVIA Centaur (Bayer Diagnostics), which utilizes direct chemiluminescent sandwich immunoassay; (3) Triage BNP (Biosite, Inc.), which utilizes single use fluorescence immunoassay; and (4) Beckman Coulter (Biosite, Inc.), which utilizes two-site chemiluminescent immuno-enzymatic assay. The Biosite Triage BNP Test is a point-of-care test with a turnaround time of 15 minutes, making it readily available for use in emergency departments and physicians' offices.

N-Terminal-ProBNP

Diagnostic cutoff: 300 pg/mL (300 ng/L)

N-terminal-proBNP (NT-proBNP) is a more stable form of BNP that correlates well with BNP in patients with heart failure, although its levels are typically higher than BNP levels. In addition, NT-proBNP levels are elevated in the elderly and, accordingly, the clinical diagnostic cutoff level for heart failure is higher in older patients. An NT-proBNP level <300 pg/mL was optimal for ruling out acute CHF, with a negative predictive value of 99%. For cut points of >450 pg/mL for patients younger than 50 years of age and >900 pg/mL for patients 50 years of age or older, NT-proBNP levels were highly sensitive and specific for the diagnosis of acute heart failure (Table 8-7).[105,126]

Other Biochemical Markers

Elevated cardiac troponin levels in patients with heart failure have been shown to be related to the severity of heart failure and worse outcomes.[127-129] In patients presenting with acute decompensated heart failure, routine measurement of troponin levels is recommended.[130] In addition to baseline troponin levels, serial troponin measurements may be useful in predicting outcomes.[131] In a recent study of patients hospitalized for acute heart failure, 60% of patients had detectable cTnT levels (>0.01 ng/mL or >0.01 mcg/L) levels and 34% had positive values (>0.03 ng/mL or >0.03 mcg/L) at baseline. Of the patients with negative troponin level at baseline, 21% had elevated cTnT levels by day 7. Positive troponin levels at baseline and conversion to detectable levels were associated with a poor prognosis.[132]

Recommendations for Measurement of Biochemical Markers

Measurement of BNP or NT-proBNP is useful (1) to support clinical decision making regarding the diagnosis of heart failure in ambulatory patients with dyspnea or in a patient with acutely decompensated heart failure, especially in the setting of clinical uncertainty, and (2) for establishing prognosis or disease severity in chronic and acute decompensated heart failure. BNP/NT-proBNP–guided heart failure therapy can be useful to achieve optimal dosing in select clinically euvolemic patients in the ambulatory care setting, but they are not well established in acute decompensated heart failure. In addition, the usefulness of serial measurement of BNP or NT-proBNP to reduce hospitalization or mortality in patients with heart failure is not well established. Measurement of cardiac troponins, as biomarkers of myocardial injury, is helpful for establishing prognosis and risk stratification in the ambulatory/outpatient and the acute setting.[2]

IMAGING STUDIES

A number of imaging modalities contribute to the diagnosis and assessment of ACS, including chest roentgenography, echocardiography, cardiac catheterization, perfusion imaging, computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET).

Chest Roentgenogram

Chest radiography taken at the initial presentation of patients with ACS provides an early estimation of the cardiac silhouette. In addition, presence and degree of pulmonary congestion indicates elevated left-ventricular end-diastolic pressure, which may result from a sizeable infarction of the left ventricle. Chest radiography is a standard study in evaluating patients presenting with symptoms suggestive of heart failure. Findings of heart failure include cardiac enlargement, vascular redistribution, interstitial and alveolar edema, peribronchial cuffing, and pleural effusions. Cardiac enlargement on chest roentgenogram in patients with heart failure is associated with a higher morbidity rate.

Echocardiography

Echocardiography is based on sound transmitted to and through the heart. Different tissues present different acoustical impedance (resistance to transmitting sound). Transthoracic echocardiography (TTE) involves sound waves from a transducer positioned on the anterior chest directed across cardiac tissues. The sound is reflected back in different frequencies, and images of cardiac anatomy are displayed on an oscilloscope or an electronic monitor.

Two-dimensional (2D) echocardiography records multiple views providing cross-sectional images of the heart. Clinical uses include anatomic assessment of the heart and functional assessment of cardiac chambers and valves. Contrast agents may be injected for better visualization of endocardial borders. M-mode, or motion-mode, records the motion of individual structures. Doppler echocardiography uses sound or frequency ultrasound to record the velocity and direction of blood and wall motion; it is based on the principle of bouncing ultrasound waves off of a moving object (e.g., red blood cells). This method permits the assessment of valvular and wall motion abnormalities.

As a noninvasive test that measures cardiac hemodynamics and filling pressures, TTE plays an important role in assessing patients with ACS presenting with apparent large infarct and hemodynamic instability. Information provided includes wall motion abnormalities to assess the extent of the infarct or the level of function of the remaining myocardium, recognition of complications such as postinfarction ventricular septal defect, and the presence of left ventricular thrombi.[133] In addition, TTE can be combined with exercise and pharmacologic stress testing to assess stress-induced structural or functional abnormalities (e.g., wall motion abnormality associated with ischemia) in patients with IHD.

Left ventricular ejection fraction is a valuable diagnostic and prognostic index in patients with failure, and it is defined as the fraction of end-diastolic volume ejected from the left ventricle during each systolic contraction. The normal range for LVEF is 55–75%. Patients may have heart failure due to diastolic ventricular dysfunction with a normal LVEF. In clinical practice, TTE is currently the modality most utilized and accepted for the evaluation of systolic and diastolic cardiac function.

Transesophageal echocardiography (TEE) involves mounting the transducer at the end of a flexible endoscope and passing it through the esophagus to position it closer to the heart. TEE provides higher resolution of the posterior cardiac structures making it ideal for viewing the atria, cardiac valves, and aorta. Clinical indications include detection of atrial appendage thrombi, native and prosthetic valvular function and morphology, cardiac masses, or thoracic aortic dissection. TEE is used for the evaluation of hemodynamically unstable patients with conditions that would yield poor TTE imaging. Appropriateness criteria for use of TTE and TEE in the evaluation of cardiac structure and function has been published.[134]

Newer and sophisticated techniques such as speckle tracking and strain rate are now utilized in 2D echocardiography to aid in evaluation of the dynamic nature of the myocardial function and provide more precise quantitative myocardial function assessment. Three-dimensional (3D) echocardiography provides improved accuracy over 2D echocardiography and is recommended for left ventricular function assessment (volume and ejection fraction).[135] 3D echocardiography is continuously being used in clinical practice and has revolutionized the field with improved imaging of anatomical details. 3D echocardiography is continuously being utilized in evaluation of cardiac defects, valvular abnormalities, intraoperative and intracardiac catheter based interventions.[136,137]

Noninvasive Stress Testing

Exercise Stress Testing

In patients with chronic stable CAD who are capable of physical exercise, myocardial perfusion imaging is used in conjunction with exercise testing. Physical exercise is performed using a graded exercise protocol on a treadmill or upright bicycle. The most widely used protocol is the Bruce protocol. Nonimaging endpoints include reproduction of anginal symptoms, exhaustion, hypertension or 10 mm Hg decrease in systolic blood pressure from baseline, ventricular arrhythmias, or severe ST-segment depression on ECG. Exercise allows for several useful measurements including the duration of exercise, total workload, maximum HR, exercise-induced symptoms, ECG changes, and blood pressure response. Limitations include patients with orthopedic, neurological, or peripheral vascular problems. Patients receiving agents that may blunt HR response to exercise (β-blockers or nondihydropyridine calcium channel blockers) may not be able to achieve the target HR necessary for diagnostic and prognostic purposes.

Pharmacologic Stress Testing

Patients who are unable to exercise may be stressed pharmacologically using either (1) vasodilating agents such as adenosine or dipyridamole or (2) positive inotropic agents such as dobutamine. Both modalities produce a hyperemic (vasodilatory response or increased blood flow) response leading to heterogeneity of myocardial blood flow between vascular areas supplied by normal and significantly stenosed coronary arteries. Heterogeneity is visualized with radionuclide myocardial perfusion agents.

Adenosine is an endogenous vasodilator. Coronary vasodilation is mediated through the activation of A_{2a} receptors. Dipyridamole blocks the cellular reabsorption of endogenous adenosine. Vasodilation, by both adenosine and dipyridamole, increases coronary blood flow in normal arteries three to five times baseline with little or no increase in blood flow to stenotic arteries. Dobutamine increases myocardial oxygen demand by increasing myocardial contractility, HR, and blood pressure. Following dobutamine administration, coronary blood flow in normal arteries is increased two to three times baseline, which is similar to that achieved with exercise. Myocardial uptake of thallium-201, a radiopharmaceutical agent, is directly proportional to coronary blood flow (see Myocardial Perfusion Imaging). Administration of the above pharmacologic agents causes relatively less thallium-201 uptake in myocardial areas supplied by stenotic arteries. Therefore, a greater difference is seen between tissue supplied by normal arteries and tissue supplied by stenotic arteries.

Cardiac Catheterization

Cardiac catheterization involves the introduction of a catheter through the femoral or radial artery, which is advanced to the heart chambers or great vessels guided by fluoroscopy. Measurements collected include intracardiac pressures, hemodynamic data, and blood flow in the heart chambers and coronary arteries.

Coronary Angiography

Coronary angiography, also referred to as *angiocardiography* or *coronary arteriography*, is a diagnostic test in which contrast media is injected into the coronary arteries. X-ray exposures of the coronary arteries are then examined to assess the location and severity of coronary atherosclerotic lesions. Therapeutic interventions or PCI may be performed during the catheterization including percutaneous transluminal coronary angioplasty and bare metal or drug-eluting stent placement.

Left Ventriculography

Left ventriculography is the injection of contrast media into the left ventricle to assess its structure and function. The test is often performed as part of a diagnostic cardiac catheterization to evaluate ventricular wall motion and measure LVEF.

Nuclear Imaging

Nuclear imaging involves the injection of trace amounts of radioactive elements that concentrate in certain areas of the heart. A gamma camera is then rotated around the patient, and multiple planar images are taken to detect the radioactive emissions and form an image of the deployment of the tracer in the different regions of the heart. Single-photon emission CT (SPECT) is the most common imaging technique. Nuclear imaging is used to assess blood flow through the heart and perfusion of the myocardium, to locate and assess severity of myocardial ischemia and infarction, and to evaluate myocardial metabolism.

Myocardial Perfusion Imaging

Thallium-201 (^{201}Tl) became available in 1974 and was the conventional radiopharmaceutical agent used until the early 1990s, at which time technetium-99m (^{99m}Tc)-labeled compounds such as ^{99m}Tc-sestamibi and ^{99m}Tc-tetrofosmin were introduced for visualization of myocardial perfusion. The imaging agents measure the relative distribution of myocardial blood flow between normal and stenotic coronary arteries.

^{201}Tl, a potassium analog is taken up by healthy functioning tissue in a manner similar to potassium. ^{201}Tl is taken up at reduced rates by ischemic myocardial tissue and is not distributed to or taken up by regions of MI. Imaging with ^{201}Tl for detection of infarction is accomplished with the patient at rest and is optimal within six hours of symptom onset. ^{201}Tl is injected intravenously and imaging is initiated 10–20 minutes after injection; imaging is repeated two to four hours later to determine whether redistribution occurred. The diagnosis of CAD must be inferred by a lack of regional myocardial uptake of the radiotracer. ^{201}Tl imaging for a perfusion defect has a sensitivity of about 90% if applied within the first 24 hours after symptom onset and falls sharply thereafter.

^{99m}Tc is an infarct-avid agent. It concentrates in necrotic myocardial tissue, presumably because it enters myocardial cells and selectively binds to calcium and calcium complexes. Abnormal intracellular uptake of calcium is a feature of irreversible cell death, which begins as early as 12 hours after MI and may persist for two weeks. ^{99m}Tc scans may be positive as early as four hours after the onset of MI symptoms. The peak sensitivity for the scan is between 48–72 hours, but it generally remains positive for up to one week post-MI. When obtained within 24–72 hours after onset of infarction, the scan has a diagnostic sensitivity of 90–95% in patients with QwMI or 38–92% in patients with NQMI. ^{99m}Tc imaging has moderate specificity, with an overall range of 60–80%.

^{201}Tl perfusion imaging is widely utilized in patients with chest pain syndrome for the diagnosis of CAD. It also can be used following a nondiagnostic or false-positive exercise stress test to determine if coronary artery atherosclerosis is the cause of symptoms. In addition, because redistribution is a marker of jeopardized but viable myocardial tissue, ^{201}Tl can be used to indicate the probable success of revascularization or angioplasty and for preoperative prognostic stratification of patients. Transient defects (redistribution) indicate hemodynamically significant coronary lesions with the risk of cardiac death.

Nuclear imaging in conjunction with stress testing provides further information on myocardial perfusion and function. ^{99m}Tc-sestamibi may be used in combination with ^{201}Tl to assess rest and stress myocardial perfusion sequentially in one day. The patient's rest study is done first with ^{201}Tl; imaging is started immediately after tracer injection and is completed within about 45 minutes. Stress testing (pharmacologically or with exercise) is begun after the rest study, and ^{99m}Tc-sestamibi is injected at peak cardiac stress.

Because ^{99m}Tc-sestamibi emits higher energy photons than ^{201}Tl, its images are not subject to cross-interference from the previously administered ^{201}Tl. Both ^{201}Tl and ^{99m}Tc-sestamibi undergo first-pass extraction from blood by myocardial cells, and both provide a stop-frame image of regional myocardial blood flow at the time of tracer injection. ^{99m}Tc-sestamibi does not leak appreciably from myocardial cells; thus, imaging may be delayed to allow blood and lung concentrations to diminish. Consequently, ^{99m}Tc-sestamibi imaging can be completed between one to four hours after tracer injection without significantly reducing diagnostic reliability.

Images are interpreted qualitatively and quantitatively and assessment of myocardial perfusion to the different areas is reported as being normal, having a defect, having a reversible defect, having a fixed defect, or having reverse redistribution. Myocardial perfusion imaging may also be used for the assessment of thrombolytic therapy effectiveness and early risk stratification of patients presenting with MI or ACS who were treated conservatively at initial presentation. ECG-gated myocardial perfusion SPECT studies enhance the interpretive confidence and accuracy and provide information critical for the diagnosis, prognosis, and management decisions, including global left ventricular function and regional wall motion and thickening.

Computed Tomography

Computed tomography (CT) involves an intense, focused electron beam that is swept along target rings by electromagnets. When the electron beam strikes the target ring, a fan of x-rays is produced and moves around the patient. CT of the heart is limited by cardiac motion, which may be overcome by gating of the CT scan with a simultaneous ECG recording. Alternatively, ultrafast CT allows scanning in real time without gating. The main advantage of CT is enhanced resolution and special definition of structures.

In recent years, rapid advancement in state-of-the-art, noninvasive cardiac imaging technology has occurred, which has been accompanied by an increased demand for its use. Cardiac CT (CCT) is useful in the assessment of cardiac structure including cardiac masses, pericardial conditions, and evaluation of aortic (e.g., dissection or aneurysm) and pulmonary disease. It is also appropriate in the evaluation of chest pain syndrome in patients with low-to-intermediate pretest probability of CAD.[138] Studies comparing CCT to other diagnostic strategies are needed to optimize use and evaluation of specific patient subsets. One study showed that CCT can be more cost efficient and provide faster diagnosis in low-risk patients presenting with chest pain than myocardial perfusion imaging.[139] Another study showed that in symptomatic patients with suspected CAD who required noninvasive testing, compared with functional testing (exercise electrocardiography, nuclear stress testing, or stress echocardiography), a strategy of initial CT did not improve clinical outcomes (composite end point of death, MI, hospitalization for UA, or major procedural complication) over a median follow-up of two years.[140]

Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) is a noninvasive imaging technique capable of detailed tissue characterization and blood flow measurements. The procedure involves placing patients in a device generating a strong magnetic field and aligning the protons of the body's hydrogen atoms relative to the magnetic field. Radio waves are pulsed through the field and force the protons to shift their orientation. When the radio waves stop, the protons return to their previous orientation, releasing energy in the form of radio waves. The waves are detected by a scanner and converted into images. The images are physiologically gated to an ECG.

During the procedure, the patients are required to remain motionless. Claustrophobic patients may not be able to undergo the procedure. Sedation may be necessary. In addition, patients with metal prostheses are unable to undergo MRI. Patients with certain implantable cardioverter-defibrillators and pacemakers are able to undergo MRI if the devices are tested and programmed to MRI-safe settings before the procedure and reprogrammed to the original settings after the procedure.

Clinical uses of cardiac MRI (CMR) include assessment of congenital, aortic, and pericardial diseases, tumors, and intravascular thrombus. Magnetic resonance imaging has become more available but restricted to some medical centers because of the cost, scan time, and need for specialized equipment and personnel. Current recommendations include the use of vasodilator perfusion CMR or dobutamine stress function CMR for the evaluation of chest pain syndrome in patients with intermediate pretest probability of IHD in the setting of uninterpretable ECG or an inability to exercise. CMR is also recommended for the evaluation of suspected coronary anomalies. There is no widely acceptable use for CMR in the clinical setting for acute chest pain at this time.[141]

Positron Emission Tomography

Positron emission tomography (PET) is a nuclear imaging technique capable of measuring myocardial blood flow and cellular metabolism of substrates such as fatty acids, glucose, and oxygen in vivo. The technique uses the properties of short-lived, positron-emitting, isotope-labeled compounds (nitrogen-13, oxygen-15, carbon-11, or fluorine-18) coupled with mathematical models of physiological function. Its most relevant clinical use for cardiovascular evaluation is detection of ischemic, but viable (or *hibernating*), myocardium that appears irreversibly necrotic by other diagnostic tests.

Blood Pool Imaging

Blood pool scintigraphy is used to evaluate ventricular wall motion and function as well as left ventricular volume. Human serum albumin or the patient's red blood cells are tagged with ^{99m}Tc and injected intravenously into the patient. A scintillation camera records the radioisotope as it passes through the ventricle. Imaging can be gated or linked with a simultaneous ECG recording. Multiple images are taken and are combined to produce a cine film permitting the evaluation of ventricular chamber size, wall motion, filling defects, and ventricular ejection fraction.

SUMMARY

The heart is a muscular pump that circulates blood to the lungs for oxygenation and throughout the vascular system to supply oxygen and nutrients to every cell in the body. Many conditions affect the heart's function including CAD, ACS, and heart failure. The classic laboratory workup for ACS includes the measurement of serum cTnI or cTnT, and, uncommonly, CK-MB. Classic ECG changes, such as T-wave inversion, ST-segment depression or elevation, and Q-wave appearance, may also be present and are useful in evaluating patients presenting with ACS. In addition to confirming an equivocal diagnosis, imaging techniques may localize and estimate the size of MIs. After an MI, LVEF may be determined for prognostic information and appropriate initiation of guideline-directed medical therapy. Measurement of BNP and CRP provides additional prognostic information. For the diagnosis and assessment of heart failure, BNP or NT-proBNP measurement is considered the gold standard test. Determination of LVEF is essential for differentiating systolic (reduced LVEF) from diastolic (preserved LVEF) heart failure and targeting therapy accordingly.

The clinician must be well informed of the various tests used to diagnose and assess patients with CAD, ACS and its potential complications, and heart failure. Knowledge of these tests and their clinical significance greatly impacts decisions regarding implementation of appropriate management strategies and preventative measures.

LEARNING POINTS

1. ***Summarize the criteria used in the assessment of patients presenting with ACS.***

   ANSWER: Three criteria are used to evaluate patients presenting with possible ACS. These are clinical presentation or symptoms, ECG changes, and cardiac biochemical markers such as cardiac troponins and CK-MB. The latter two are used to distinguish between a diagnosis of UA/NSTEMI and STEMI. Classic ECG changes include ST-segment elevation, consistent with STEMI, or ST-segment depression or T-wave inversion, consistent with NSTEMI. Elevated levels of plasma cardiac biomarkers are consistent with infarction and distinguish UA from NSTEMI.

2. ***Discuss the release kinetics of cardiac specific troponins and recommendations for measurement of this laboratory test in patients presenting with chest pain.***

   ANSWER: cTnI and cTnT levels are detectable above the upper reference limit by three hours from the onset of symptoms. Mean time to peak elevation levels without reperfusion therapy is 24 hours for cTnI and 12 hours to 2 days for cTnT. Due to continuous release from injured myocytes, cTnI levels may remain elevated for 5–10 days after an MI versus 5–14 days for cTnT. Levels are obtained at initial presentation of patients with chest discomfort and repeated three to six hours later to confirm the diagnosis of MI.

3. ***Define the LVEF classification of heart failure.***

   ANSWER: Heart failure is classified based on the LVEF to one of four types: (1) HF*r*EF (LVEF of ≤40%, also referred to as *systolic heart failure*); (2) HF*p*EF (LVEF of ≥50%, also referred to as *diastolic heart failure*); (3) HF*p*EF, borderline (LVEF between 41% and 49%); and (4) HF*p*EF, improved (current LVEF of >40% with a prior history of HF*r*EF).

4. ***Define the utility of BNP levels in the clinical assessment of patients presenting with heart failure.***

   ANSWER: The BNP levels are a good marker of left ventricular dysfunction and a strong marker to predict morbidity and mortality in patients with heart failure. In conjunction with the standard clinical assessment, BNP is used to establish or exclude the diagnosis of heart failure in patients presenting to emergency departments for evaluation of acute dyspnea. Serum BNP levels correlate with the clinical severity of heart failure as assessed by NYHA classification.

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QUICKVIEW | Troponins I and T

PARAMETER	DESCRIPTION	COMMENTS
Common reference ranges	Troponin I: ≤0.02 ng/mL (≤0.02 mcg/L)	Assay dependent; Table 8-3
	Troponin T: <0.1 ng/mL (<0.1 mcg/L)	Assay dependent
Critical value	Troponin I: ≥0.3 ng/mL (≥0.3 mcg/L)	Assay dependent; Table 8-3
	Troponin T: ≥0.1 ng/mL (≥0.1 mcg/L)	Assay dependent
Inherent activity	Yes	Regulates calcium-medicated interaction of actin and myosin
Location	Cardiac and skeletal muscle	Cardiac troponins I and T and skeletal muscle troponins I and T have different amino acid sequences
Cause of abnormal values		
High	MI; myocardial ischemia produces mild elevations of troponin levels, which indicate increased risk for cardiac events	
Low	Normal finding	No lower limit for normal
Signs and symptoms		
High	Chest pain, nausea, vomiting, diaphoresis	Decreased or increased HR and BP, anxiety, and confusion, depending on MI size, location, and duration
Low	None	Does not cause signs and symptoms
After MI, time to...		
Initial elevation	4 hr	Time course studies of release needed
Peak values	12 hr–2 days	
Normalization	5–14 days	
Causes of spurious results	Table 8-4	

BP = blood pressure; HR = heart rate; MI = myocardial infarction.

QUICKVIEW | CK-MB

PARAMETER	DESCRIPTION	COMMENTS
Common reference ranges		
Adults	≤6 ng/mL (≤6 mcg/L)	Assay dependent
	<3–6% of total CK	
Critical value	>6 ng/mL (>6 mcg/L)	Assay dependent
	>5% of total CK	
Inherent activity	Yes	Catalyzes transfer of high-energy phosphate groups
Location		
Production	Primarily cardiac muscle	Release from traumatized skeletal muscle can be incorrectly interpreted as cardiac in origin
Storage	Small amounts in skeletal muscle	
Secretion/excretion	Excreted via glomerular filtration	Eliminated at slightly faster rate than total CK
Causes of abnormal values		
High	MI	
Low	Not significant	No lower limit for normal
Signs and symptoms		
High level	AMI: chest pain, nausea, vomiting, diaphoresis	Decreased or increased HR and BP, anxiety, and confusion, depending on MI size, location, and duration
Low level	None	Does not cause signs and symptoms
After MI, time to...		
Initial elevation	3–12 hr	
Peak values	12–24 hr	
Normalization	2–3 days	
Causes of spurious results	Table 8-6	

BP = blood pressure; CK = creatine kinase; HR = heart rate; MI = myocardial infarction.

QUICKVIEW | BNP/NT-proBNP

PARAMETER	DESCRIPTION	COMMENTS
Common reference ranges		
Adults	BNP: <100 pg/mL (<100 ng/L)	
	NT-proBNP: <300 pg/mL (<300 ng/L)	
Critical value	BNP: >500 pg/mL (>500 ng/L)	
	NT-proBNP: >450 pg/mL in patients <50 years of age; >900 pg/mL in patients 50–75 years of age; >1800 pg/mL in patients >75 years of age	Affected by age, gender, renal function, and obesity
Inherent activity	Yes	Diuretic, natriuretic, and vascular smooth muscle-relaxing effects
Location		
Production/storage	Ventricular myocyte	Released in response to increased ventricular wall tension
Secretion/excretion	BNP: enzymatic degradation via endopeptidase and natriuretic peptide receptor-mediated endocytosis	
	NT-proBNP: renal elimination	
Causes of abnormal values		
High	Heart failure	
Low	Not significant	
Signs and symptoms		
High level	Shortness of breath, pulmonary and peripheral edema	
Low level	None	
Causes of spurious results	Pulmonary embolism, pulmonary hypertension, pericarditis, sepsis	

BNP = B-type natriuretic peptide; NT-proBNP = N-terminal-proBNP.

9

LIPID DISORDERS

Jill S. Borchert and Kathy E. Komperda

OBJECTIVES

After completing this chapter, the reader should be able to

- **List primary and secondary causes of dyslipidemia**
- **Outline the physiology of lipid metabolism and correlate lipid levels to the risk of atherosclerotic cardiovascular disease**
- **Calculate low-density lipoprotein when provided with total cholesterol, high-density lipoprotein, and triglyceride values**
- **Given a case study, interpret laboratory results from a lipid profile and discuss how they should guide treatment choices**

Dyslipidemia, or an abnormal serum lipid profile, is a major risk factor in the development of atherosclerotic cardiovascular disease (ASCVD).[1] Clinical manifestations of ASCVD include acute coronary syndrome, myocardial infarction (MI), angina, coronary revascularization (e.g., coronary bypass, percutaneous coronary intervention), stroke and transient ischemic attack (TIA).[2] Over 85.6 million adults in the United States are affected by cardiovascular disease and one in three American adults has at least one cardiovascular disease.[3] Cardiovascular disease is a leading cause of death and preventative efforts are essential to decrease associated morbidity and mortality.

Efforts in the management of dyslipidemia have contributed to a decline in cardiovascular morbidity and mortality.[1] Nonetheless, more than half of adults in the United States fail to meet the cholesterol level for ideal cardiovascular health set by the American Heart Association (AHA).[3] Further, approximately one quarter of American adults have not been screened for dyslipidemia with a lipid panel. Practitioners are being asked to assess the lipid panel in an effort to decrease overall cardiovascular risk. It is estimated that over 50 million adults in the United States are eligible for statin therapy to target a reduction in ASCVD risk.

This chapter primarily covers the physiology of cholesterol and metabolism of triglycerides (TGs), their actions as part of lipoproteins, disorders of lipids and lipoproteins, and consequences of elevated lipid levels. The effects of diet, exercise, and drugs on these lipid values are also discussed. A detailed interpretation of test results and drug therapy to reduce cardiovascular risk is beyond the scope of this chapter, but references provide additional information.[1,2,4]

PHYSIOLOGY OF LIPID METABOLISM

Lipids are an essential component of several biological processes. The major plasma lipids are cholesterol, TGs, and phospholipids. Cholesterol serves as a structural component of cell wall membranes and is a precursor for the synthesis of steroid hormones and bile acids.[5,6] TGs, the esterified form of glycerol and fatty acids, constitute the main form of lipid storage in humans and serve as a reservoir of fatty acids to be used as an energy source for the body.[5] Phospholipids are lipid molecules that contain a phosphate group. Like cholesterol, phospholipids become constituents of cell wall membranes. Both cholesterol and TGs are hydrophobic, while phospholipids are hydrophilic.[7] Cholesterol and TGs are surrounded by proteins and phospholipids to form lipoproteins. These lipoproteins are more water soluble and can then be transported in the body. Given that the laboratory measurement of plasma lipids is the sum of cholesterol and TGs circulating in the different lipoproteins, an understanding of the synthesis and metabolism of these lipoproteins is necessary for proper diagnosis and treatment of dyslipidemia to reduce overall cardiovascular risk.

Cholesterol and TGs can be absorbed from the diet (exogenous) or synthesized in the body (endogenous) (**Figure 9-1**), while phospholipids are primarily synthesized in the body.[5] Cholesterol is continuously undergoing synthesis, degradation, and recycling. Approximately half of cholesterol consumed in the diet is absorbed; however, dietary cholesterol contributes relatively little to serum cholesterol levels.[5] Exogenous TGs are transported from the intestine to the systemic circulation via chylomicrons,

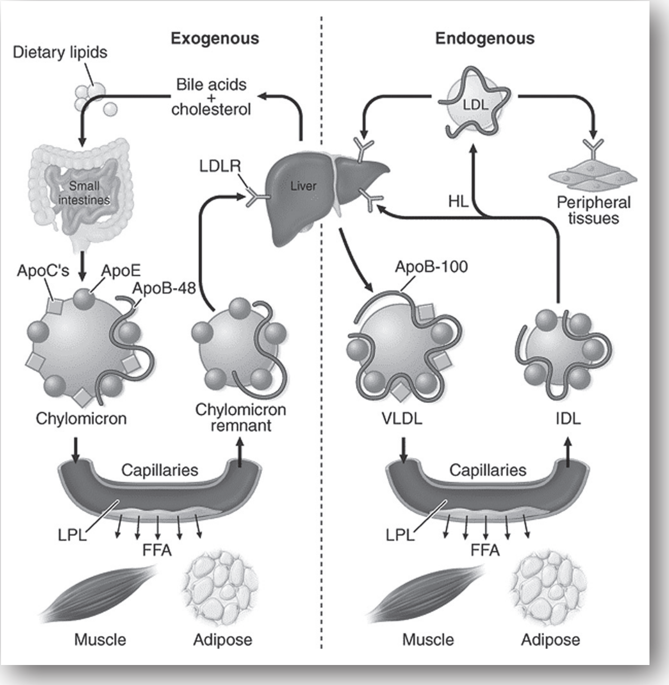

FIGURE 9-1. Lipid metabolism. Apo = apolipoprotein; FFA = free fatty acid; HL = hepatic lipase; IDL = intermediate-density lipoprotein; LDL = low-density lipoprotein; LDLR = low-density lipoprotein receptor; LPL = lipoprotein lipase; VLDL = very low-density lipoprotein. *Source:* Reprinted with permission from Kasper DL, Fauci AS, Hauser SL et al., eds. Harrison's principles of internal medicine. 19th ed. New York: McGraw-Hill Education; 2015. Copyright © McGraw-Hill Education. All rights reserved.

which are TG rich lipoproteins.[6] Endogenous production of cholesterol, TGs, and phospholipids primarily occurs in the liver and intestinal tract.[5,6] The majority of serum cholesterol is derived from cholesterol synthesized in the liver. Intestinal cholesterol absorption, hepatic cholesterol synthesis, and excretion of cholesterol and bile acids regulate serum cholesterol concentrations (Figure 9-1).[4,5] Most cholesterol synthesis occurs during the night.[8] The rate-limiting step in cholesterol synthesis is the conversion of hepatic hydroxymethylglutaryl-coenzyme A (HMG-CoA) to mevalonic acid.[4,6] This conversion is catalyzed by the enzyme HMG-CoA reductase.[4] An inhibitory feedback mechanism modulates cholesterol synthesis.[5] The presence of cholesterol in hepatic cells leads to decreased biosynthesis of cholesterol. Conversely, when hepatic cholesterol concentrations decrease, there is a resulting increase in hepatic cholesterol biosynthesis. However, the feedback inhibition mechanism is inadequate in preventing a rise in serum cholesterol levels in the presence of a diet high in calories and saturated fat.

Cholesterol, TGs, and phospholipid molecules complex with specialized proteins (apolipoproteins) to form lipoproteins, the transport form in which lipids are measured in the blood.[4,5,7] As lipids are insoluble in aqueous plasma, they are formed into complexes with an outer hydrophilic coat of phospholipids and proteins and an inner core of fatty cholesterol and TGs. The apolipoproteins not only serve to support the formation of lipoproteins, but they also mediate binding to receptors and activate enzymes in lipoprotein metabolism. All lipoproteins contain phospholipids, TGs, and esterified and unesterified cholesterol in varying amounts. There are many ways to classify these lipoproteins, but most frequently lipoproteins are classified by their density, size, and major apolipoprotein composition. **Table 9-1** summarizes the characteristics of the five major classes of lipoproteins.[4,5] The major apolipoproteins listed in Table 9-1 are simply a summary of the apolipoproteins involved in lipoprotein formation. Of note, atherogenic lipoproteins contain apolipoprotein B (apo B), while high-density lipoprotein (HDL) does not. This will be discussed further in the setting of interpretation and clinical significance of non-HDL levels. Other apolipoproteins include apoA-II, apoA-IV (primarily associated with HDL); apoC-I, apoC-II, and apoC-III (primarily associated with TG-rich lipoproteins); apoE (TG-rich lipoproteins); and apo(a), which is associated only with lipoprotein(a) [Lp(a)].[4]

All of the major lipoproteins have a role in cholesterol metabolism and transport in the body.[4,6] Chylomicrons, which are TG-rich, deliver TGs from the gastrointestinal tract to the muscle and adipose tissue where lipoprotein lipase (LPL) releases fatty acids and glycerol. After this process, the chylomicron is no longer TG rich and is referred to as a chylomicron remnant and is delivered to the liver. The liver can export cholesterol and other TGs in the form of very low-density lipoproteins (VLDL) into the circulation. Similar to

TABLE 9-1. Characteristics of Lipoproteins[4,5]

LIPOPROTEIN	SIZE	DENSITY	MAJOR APOLIPOPROTEIN	ORIGIN	COMMENTS
Chylomicrons and chylomicron remnants	Largest	Least	ApoB-48	Intestines	Primarily TGs
VLDL	↑	↓	ApoB-100	Liver and intestines	Primarily TGs
IDL or remnants			ApoB-100	Chylomicrons and VLDL	Transitional forms
LDL			ApoB-100	End-product of VLDL	Major carrier of cholesterol
HDL	Smallest	Most	ApoA-I	Intestines and liver	Removes cholesterol from atherosclerotic plaques in arteries

HDL = high-density lipoprotein; IDL = intermediate-density lipoprotein; LDL = low-density lipoprotein; TGs = triglycerides; VLDL = very low-density lipoprotein.

chylomicrons, VLDL are predominantly TG rich but have a higher cholesterol composition than chylomicrons (5 mg of TGs per 1 mg of cholesterol).[4] Once in circulation, VLDL undergoes the same degradation as chylomicrons via LPL.[4,6] This LPL activity then converts VLDL particles to intermediate-density lipoproteins (IDL) and eventually low-density lipoproteins (LDL). LDL typically carries the largest portion of cholesterol in the body. The liver degrades the majority of circulating LDL; however, other tissues can take up a small portion of LDL that provides necessary cholesterol for cell membrane and steroid synthesis. LDL in general is considered to be atherogenic and has been a focus of dyslipidemia management. The last major lipoprotein is HDL, and unlike LDL, it is typically considered to be protective against atherosclerosis via reverse cholesterol transport (**Figure 9-2**). One role of HDL is to acquire excess cholesterol from degraded VLDL and the periphery. HDL undergoes an enzymatic reaction via lecithin cholesterol acyltransferase to become HDL cholesteryl ester, which is then selectively taken up by the liver and targeted for excretion via bile. In addition, the cholesteryl ester transfer protein can transfer cholesteryl ester from HDL to VLDL, IDL, and LDL, which results in less dense lipoproteins that can be taken up by the liver more easily.

Elevated cholesterol is a known contributor to the development of atherosclerosis. Proper diagnosis and treatment of dyslipidemia can be an important preventative strategy. Numerous trials of effective treatment have demonstrated reductions in cardiovascular events, stroke, and total mortality in patients with a prior history of ASCVD (secondary prevention) and in patients with asymptomatic dyslipidemia (primary prevention).[9-15]

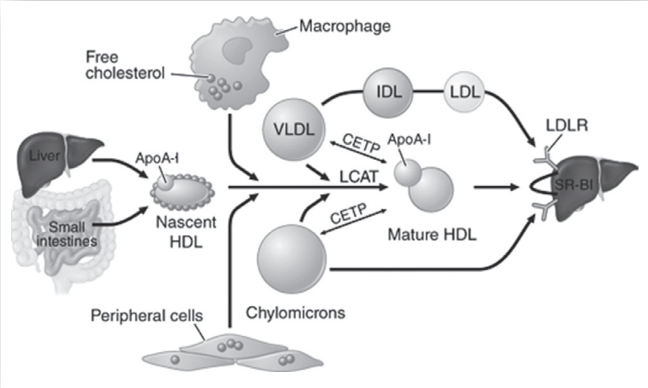

FIGURE 9-2. HDL metabolism and reverse cholesterol transport. Apo = apolipoprotein; CETP = cholesteryl ester transfer protein; HDL = high-density lipoprotein; IDL = intermediate-density lipoprotein; LCAT = lecithin-cholesterol acyltransferase; LDL = low-density lipoprotein; LDLR = low-density lipoprotein receptor; SR-BI = scavenger receptor class BI; VLDL = very low-density lipoprotein. *Source:* Reprinted with permission from Kasper DL, Fauci AS, Hauser SL et al., eds. Harrison's principles of internal medicine. 19th ed. New York: McGraw-Hill Education; 2015. Copyright © McGraw-Hill Education. All rights reserved.

Primary Lipid Disorders

Dyslipidemias, or abnormal concentrations of any lipoprotein type, are classified by etiology into primary or secondary disorders. *Primary lipid disorders* are caused by genetic defects in the synthesis or metabolism of the lipoproteins. **Table 9-2** shows

TABLE 9-2. Classification of Selected Primary Dyslipidemias[4,16-18]

PRIMARY LIPID ABNORMALITY	PRIMARY DYSLIPIDEMIA	SELECTED FEATURES[a]	CLINICAL MANIFESTATIONS
Increased LDL	Familial hypercholesterolemia	LDL receptor defect, LDL 200–500 in heterozygous, 500–800 in homozygous; prevalence 1 in 500	Tendinous xanthomas, premature ASCVD
	Familial defective ApoB-100	ApoB-100 mutation impairs LDL binding, LDL 200–400 in heterozygous, 500–800 in homozygous; prevalence 1 in 2000	Tendinous xanthomas, premature ASCVD
Increased TGs	Familial hypertriglyceridemia	Increases TG-rich VLDL particles, TGs 200–1000, occurs in 5–10% of population	Often asymptomatic unless associated with metabolic syndrome
	Familial apoC-II deficiency	Autosomal recessive disorder, severe fasting hypertriglyceridemia	Eruptive xanthomas, hepatosplenomegaly, pancreatitis
	Lipoprotein lipase deficiency	Mutation in lipoprotein lipase gene or its cofactor, TGs 2000–25,000 mg/dL	Eruptive xanthomas, hepatosplenomegaly, pancreatitis
Increased TGs and cholesterol	Familial combined hyperlipidemia	Overproduction of ApoB, increases production of VLDL, occurs in 1–2% of population, elevations in LDL, TGs, and TC, but degree varies widely	Premature ASCVD
	Familial dysbetalipoproteinemia	ApoE mutation, TGs 300–400 mg/dL	Palmar and plantar xanthomas, premature ASCVD, peripheral vascular disease

ASCVD = atherosclerotic cardiovascular disease; HDL = high-density lipoprotein; LDL = low-density lipoprotein; TC = total cholesterol; TGs = triglycerides; VLDL = very low-density lipoprotein.

[a] TGs, LDL, HDL, and TC in mg/dL. Conversion factor for LDL, HDL, and TC in International System (SI) units (millimoles per liter) is 0.02586. Conversion factor for TGs in SI units (millimoles per liter) is 0.01129.

the characteristics of the major primary dyslipidemias.[4,16,17,19] Historically, familial dyslipidemias were categorized by the Fredrickson electrophoresis profile of lipoproteins. More recently, clinicians have shifted to classification by the primary lipid parameter affected.[16] Primary lipid disorders rarely occur alone, and it is unlikely for a genetic predisposition to be the sole cause of a lipid disorder. Clinically, other causes, such as diet or medications, should be considered and minimized in all patients.

Secondary Lipid Disorders

Secondary lipid disorders are precipitated by other disease states, medications, or lifestyle (**Table 9-3**).[4,20-22] When a secondary cause is likely responsible for the lipid abnormality, treatment of the underlying cause should be strongly considered.

Common disease-related causes of dyslipidemia are diabetes and thyroid disorders. Patients with well-controlled type 1 diabetes mellitus typically do not have abnormal lipid levels; however, patients with type 2 diabetes mellitus may present with elevated TG levels, decreased HDL cholesterol levels, and increased levels of small, dense LDL.[23] These abnormalities may persist despite adequate glycemic control, but optimization of glycemic control is still considered an important step. LDL cholesterol concentrations and, in some cases, TG levels increase in hypothyroidism.[4] In addition to these endocrine disorders, chronic kidney disease and liver disorders should be excluded.[4] Alterations in lipid concentrations are dependent on the type of renal disorder present. For example, patients with chronic kidney disease present with elevations in TGs, while lipid profiles in patients with nephrotic syndrome will be characterized by markedly elevated LDL cholesterol and TGs.[4,20] Different liver disorders also have varying effects on lipid profiles.[4] It is recommended that secondary causes are excluded by patient history, physical examination, and laboratory data. Laboratory tests such as fasting blood glucose, thyroid-stimulating hormone, serum creatinine, urinalysis for proteinuria, and alkaline phosphatase are useful to exclude common secondary causes of dyslipidemia.

In drug-induced dyslipidemia, withdrawal of the precipitating medication usually leads to a reversal of secondary dyslipidemia. Antihypertensive agents are frequently administered to patients with cardiovascular risk. Nonselective β-blocking agents except carvedilol, which also has α1-adrenergic receptor blocking activity, may increase TG concentrations and reduce HDL cholesterol concentrations.[22] Thiazide diuretics increase total cholesterol (TC), LDL cholesterol, and TG concentrations. Thiazide effects on the lipid panel are most pronounced with higher dosages; therefore, the use of low doses is recommended. The effects of β-blockers and diuretics may be short-term, with a return to baseline levels at one year.[24] In contrast, other commonly used antihypertensive agents have no clinically significant effects on the lipid profile. Although it is important to realize the effect of antihypertensive agents

TABLE 9-3. Secondary Causes of Dyslipidemia and Major Associated Changes in Lipoprotein Component[4,20–22]

DISORDER OR CONDITION[a]	DRUG OR DIET[a]
Anorexia (↑LDL)	Alcohol (↑TGs)
Chronic kidney disease (↑LDL, ↑TGs)	Anabolic steroids (↑LDL, ↓HDL)
Cigarette use (↓HDL)	Atypical antipsychotics (↑TGs, ↓HDL)
Diabetes mellitus (↓HDL, ↑TGs)	β-blockers (↑TGs, ↓HDL)
Glycogen storage disease (↑TGs)	Combined contraceptives[b]: oral, vaginal ring (↑TGs)
Hypothyroidism (↑LDL, ↑TGs)	Corticosteroids (↑LDL, ↑TGs)
Liver failure (↓LDL, ↓TGs)	Cyclosporine (↑LDL, ↑TGs)
Metabolic syndrome (↑TGs)	Diet high in carbohydrates (↑TGs)
Nephrotic syndrome (↑LDL, ↑TGs)	Diet high in saturated or trans fats (↑LDL)
Obesity (↓HDL, ↑TGs)	Efavirenz (↑LDL, ↑TGs)
Obstructive liver disease (↑LDL)	Estrogens, oral (↑TGs, ↑HDL, ↓LDL,)
Polycystic ovary syndrome (↑LDL, ↑TGs)	Estrogen-receptor modulators (↓LDL, ↑TGs)
Pregnancy (↑ LDL, ↑TGs)	Interferons (↑TGs)
Sedentary lifestyle (↑TGs)	Isotretinoin (↑LDL, ↓HDL, ↑TGs)
Weight gain (↑LDL, ↑TGs)	Progestins (↑LDL, ↓HDL, ↓TGs)
	Protease inhibitors (↑TGs)
	Propofol (↑TGs)
	Sirolimus (↑LDL, ↑TGs)
	Thiazide diuretics (↑LDL, ↑TGs)

HDL = high-density lipoprotein; LDL = low-density lipoprotein; TGs = triglycerides.

[a] ↑ = increase; ↓ = decrease.

[b] Effect on HDL and LDL depends on specific components.

on the lipid profile, agents that adversely affect the lipid profile are not contraindicated in patients with dyslipidemia. Careful consideration of patient-specific factors is warranted.

Other drug classes have been implicated as sources of lipid abnormalities; however, effects on the lipid panel should not be considered a class effect for these medications. Atypical antipsychotics are known to cause lipid abnormalities with olanzapine possessing the greatest potential to increase LDL cholesterol, TC, and TG levels.[25] Other atypical antipsychotics have a more variable effect including beneficial effects on the lipid panel, such as ziprasidone. Similar variability has been seen among oral contraceptives, immunosuppressive drugs, and protease inhibitors. Various oral contraceptives affect lipoproteins differently. Combination oral contraceptives increase TG concentrations. Effects on LDL and HDL are variable, depending on oral contraceptive components.[22] Oral contraceptives with second-generation progestins (e.g., levonorgestrel) that have strong androgenic properties may increase TG and LDL cholesterol levels and decrease HDL cholesterol levels. However, combined oral contraceptives with third-generation progestins (e.g., desogestrel) do not cause unfavorable effects on HDL and LDL cholesterol levels but may increase TGs. Immunosuppressive drugs such as cyclosporine, sirolimus, and corticosteroids adversely affect the lipid profile, but tacrolimus does not impact the lipid profile with the same magnitude and mycophenolate mofetil has no effect.

Protease inhibitors are known to primarily cause an increase in TG levels but may also increase LDL.[21] Ritonavir-boosted regimens of lopinavir and fosamprenavir seem to have the greatest impact and switching to atazanavir or ritonavir-boosted atazanavir causes improvements in the lipid profile. Lipid abnormalities have also been identified with other antiretroviral therapies, including the nucleoside reverse transcriptase inhibitor abacavir, the non-nucleoside reverse transcriptase inhibitor efavirenz, and the integrase inhibitor elvitegravir. On the other hand, tenofovir has been associated with improvements in the lipid profile. For this reason, tenofovir may be considered as part of an initial therapy regimen in a patient with dyslipidemia. Although drug-associated adverse effects on the lipid profile have not been directly correlated with increased risk for ASCVD, it is important to assess these effects in considering laboratory data and the appropriate treatment plan for the patient.

Lifestyle also may affect lipoprotein concentrations. Besides contributing to ASCVD risk, obesity and cigarette smoking cause an increase in serum TGs and a decrease in HDL cholesterol.[20] Lifestyle modifications including smoking cessation, physical activity, heart-healthy dietary patterns and maintenance of a healthy weight aid in reducing ASCVD risk and atherogenic lipid levels.[2,20] A diet that is high in saturated fats and trans fatty acids increases LDL cholesterol levels. Diets low in saturated fats and low in percent of calories from trans fatty acids are recommended to reduce risk of ASCVD.[19] Popular low-carbohydrate diets favorably change TGs and HDL cholesterol, but they may increase LDL cholesterol levels.[26] Light-to-moderate alcohol intake (one to two glasses of beer or wine or 1–2 ounces of liquor per day) increases HDL and is associated with lower mortality from ASCVD as compared with abstention from alcohol.[18,27] Because evidence to date is epidemiologic in nature, alcohol is not recommended until data from controlled clinical trials are available.[27] The actual effect of alcohol consumption on TGs is variable.[19] It appears that light alcohol consumption may be associated with little to no change in TG levels. However, TG levels increase as alcohol consumption increases, particularly when excess alcohol is consumed with a meal high in saturated fat.[27]

LABORATORY TESTS FOR LIPIDS AND LIPOPROTEINS

Laboratory tests can be used to assess the concentrations of various lipids in the blood, making ASCVD risk assessment possible. Identification of patients at risk for ASCVD is a two-part process. First, a laboratory assessment of the lipid profile must occur. Second, an assessment of the overall ASCVD risk, including an assessment of additional cardiovascular risk factors, must occur. Multiple guidelines regarding dyslipidemia screening and management are available.[2,20,28] A level of inconsistency between the guidelines exists and a detailed summary of the recommendations is beyond the scope of this chapter; however, key messages regarding lipid management will be presented.

The two major guidelines include the 2013 American College of Cardiology/American Heart Association (ACC/AHA) Guideline on the Treatment of Blood Cholesterol to Reduce Atherosclerotic Cardiovascular Risk in Adults and the National Lipid Association (NLA) Recommendations for Patient-Centered Management of Dyslipidemia.[2,20] Both recommend acquiring a lipid panel at least every five years for any adult patient over the age of 20. This monitoring could be repeated more often if lipid values are in an undesirable range or the clinician determines the patient's ASCVD risk factors have changed.[1] The standard lipid panel includes TC, TGs, HDL, and calculated LDL. This is only one component of the overall ASCVD risk assessment and should be done in conjunction with a review of other established risk factors, including age, gender, blood pressure, diabetes, and smoking status.[28] Screening recommendations differ for pediatric patients. The Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents recommends a fasting lipid panel for children between the ages of 2 and 8 if the child has a positive family history for premature cardiovascular disease, a parent with known dyslipidemia, or the child has cardiovascular risk factors, such as hypertension, diabetes, or elevated body mass index.[29] In addition, universal screening is recommended in all pediatric patients between the ages of 9 and 11. Either a fasting lipid panel or a nonfasting sample, focusing on non-HDL cholesterol and HDL cholesterol, can be used for universal screening in this age group. No routine screening is recommended during puberty because levels may fluctuate. Reference ranges and treatment strategies for pediatric patients differ from the adult population. A review of such pediatric recommendations is beyond the scope of this chapter.

The lipid panel is ideally performed under fasting conditions; however, if it is a nonfasting panel then the TC and HDL cholesterol levels are unaffected as eating causes clinically insignificant differences in these two levels. A typical sample is collected following a 9-hour to 12-hour fast, which would include the avoidance of food and beverages with any caloric content.[30] This recommendation is based on the increase in TGs seen with a fat tolerance test, which typically includes a much higher fat intake than average meals.[31] However, this recommendation is being questioned as more recent literature suggests the change in lipid levels after a recent meal is less significant.[31,32] In general, it has been estimated that compared to the fasting state, the nonfasting LDL cholesterol level is up to 10% lower and the nonfasting TG level is up to 20% higher.[32] Given this new information, it is feasible to consider a nonfasting lipid panel in a patient especially if the burden of obtaining the lipid panel under fasting conditions may delay and or prevent lipid testing.[33] However, if TG measurement is the focus of the laboratory test or if TGs are >400 mg/dL in a nonfasting sample, then a fasting lipid panel would be required.

A number of laboratory factors may cause deviations in the lipid values obtained. Ideally, the patient should remain seated five minutes prior to phlebotomy and tourniquet application should be limited to less than one minute to avoid hemoconcentration, which may cause falsely elevated lipid levels.[30,34] Lipid panels can be drawn as either a serum or plasma sample for lipid measurements with serum being the preferred method of sampling.[35] Serum samples are collected in collection tubes without anticoagulant; plasma samples are collected in tubes with an anticoagulant. Historically, ethylenediaminetetraacetic acid was the anticoagulant of choice, but its routine use should be discouraged because it may affect analysis. Plasma concentration lipid values are approximately 3% lower than those values associated with serum measurements.[30]

Patient-specific factors may also interfere with the lipid panel results.[30] Vigorous physical activity (within the last 24 hours), pregnancy, recent weight loss, and acute illness result in levels that are not representative of the patient's usual value. For some of these circumstances (e.g. pregnancy and bacterial/viral infection), it may be beneficial to wait several weeks to obtain a lipid panel. For other circumstances, such as in the setting of acute coronary syndrome, a lipid panel should not be delayed. Measurement of plasma lipids in the setting of acute coronary syndrome usually provides LDL values that are lower than baseline 25 to 48 hours after an event.[36] The LDL values may continue to be decreased for weeks following the event. Although these values are not representative of baseline values, the recommendations are to use these values to guide initiation of LDL-lowering therapy to reduce cardiovascular risk and continue to follow lipids postdischarge.[37,38]

Unique characteristics of lipid laboratory values is that "normal" ranges are not determined by reference studies of normal subjects as most other laboratory values. Values (for TC, LDL, and TGs) below a certain value or values (for HDL) above a certain value have been identified based on epidemiological studies to determine ideal levels for decreasing cardiovascular disease risk.[34] Methods used to assay lipid panels vary among institutions. It is important to become familiar with the method of lipid profile measurement used by the laboratory that the clinician uses regularly.

Total Cholesterol

For adults >20 years[39]:
desirable, <200 mg/dL (<5.17 mmol/L);
borderline high, 200–239 mg/dL (5.17–6.18 mmol/L);
or high, ≥240 mg/dL (≥6.20 mmol/L)

Current recommendations are to use the other components of the lipid panel (e.g., LDL) instead of *total cholesterol* (TC) to guide patient care decisions.[1,2] However, TC will still be reported as part of a lipid panel, and the desirable levels included on laboratory reports are typically those from previous versions of cholesterol guidelines.[39]

Triglycerides

For adults >20 years[1]:
normal, <150 mg/dL (<1.70 mmol/L);
borderline high, 150–199 mg/dL (1.70–2.25 mmol/L);
high, 200–499 mg/dL (2.26–5.64 mmol/L);
or very high, ≥500 mg/dL (≥5.65 mmol/L)

Disorders leading to hypertriglyceridemia involve dysregulation of chylomicrons and VLDL. Chylomicrons and IDL are present only in postprandial or pathological states, while VLDL, LDL, and HDL are present in the fasting state.[30] *Triglycerides* (TGs) in the form of chylomicrons appear in the plasma soon after eating and are typically eliminated within six to nine hours after a meal. If chylomicrons persist 12 hours postprandially, this indicates an abnormal state. An overnight fast is recommended prior to measurement. As previously discussed, a typical meal does not cause a clinically significant impact on TG measurement. However, a high-fat meal (>50 g of fat) may produce a clinically significant 50% increase in TGs.[18] If a nonfasting panel is drawn, it is important to inquire about a patient's eating pattern prior to testing and consider retesting in two to four weeks if a nonfasting TG level exceeds 200 mg/dL. As a secondary disorder, hypertriglyceridemia is associated with obesity, uncontrolled diabetes mellitus, liver disease, alcohol ingestion, and uremia. Combination oral contraceptives, corticosteroids, some antihypertensive agents, protease inhibitors and isotretinoin may also elevate TG concentrations (Table 9-3).

Hypertriglyceridemia is an established risk factor for ASCVD.[1,18] In addition to increased risk of cardiovascular disease, very high TG concentrations (≥500 mg/dL or ≥5.64 mmol/L) and especially those concentrations ≥1000 mg/dL or 11.29 mmol/L may precipitate pancreatitis. Patients with pancreatitis may have intermittent episodes of epigastric pain due to recurrent pancreatic inflammation. In patients with very high TGs (concentrations ≥500 mg/dL or ≥5.64 mmol/L), the initial goal of therapy is to prevent pancreatitis through dietary modifications, alcohol avoidance, weight reduction, increased physical activity, and drug therapy. Dietary modification includes an avoidance of trans fats and reduction in

MINICASE 1

Hypertriglyceridemia

James F., a 48-year-old male, presents to the clinic to review laboratory results drawn recently for routine screening. His past medical history is significant for hypertension and obesity. He has no premature family history of ASCVD. Daily medications include lisinopril 40 mg and hydrochlorothiazide 25 mg. His diet primarily consists of processed foods and significant amounts of carbohydrates. He denies any history of tobacco use but does report drinking two to three beers most nights of the week. Physical activity is minimal beyond general daily activities. James F. has no physical complaints. He is 6'0" and 300 lb. Laboratory results are as follows: TC 175 mg/dL, TGs 689 mg/dL, and HDL 41 mg/dL. The laboratory results were drawn at 8:00 a.m.

QUESTION: How should the lipid results be interpreted? What should be done next?

DISCUSSION: The first step should be to confirm with the patient if the laboratory specimens were obtained when the patient was fasting. TGs are affected by recent food intake and the impact varies depending on the fat content of the meal. If a typical low-fat meal is ingested prior to laboratory measurements, then the effect is clinically insignificant. However, if the meal contains >50 g fat, which can be found in certain fast-food meals, then the TG levels could be increased by as high as 50%.[18] If a patient fails to fast and the TG levels are >200 mg/dL upon screening, a repeat fasting lipid panel to confirm elevated TG levels should be ordered. This patient confirms the lipids were drawn in the fasting state. An LDL value is unavailable because LDL only can be calculated with the Friedewald formula when TGs are <400 mg/dL, and it is infrequently directly measured.

This patient has very high TGs with a TG level >500 mg/dL; therefore, TG-lowering is the primary target of therapy.[1] Very high TG levels, especially those >1000 mg/dL, are associated with an increased risk of pancreatitis. Despite no symptoms or physical signs of pancreatitis, he should take immediate steps to reduce his risk. To prevent acute pancreatitis, TGs should be lowered through lifestyle modifications including dietary changes, alcohol avoidance, weight loss, and exercise. Dietary modifications include a reduction in saturated and trans fat intake without an increase in carbohydrates.[18] His current diet is high in saturated fat and carbohydrates; this is an established secondary cause of hypertriglyceridemia. Abstention from all alcohol is important to minimize the risk of pancreatitis. Because diabetes or metabolic syndrome is a common secondary cause of hypertriglyceridemia, a fasting glucose level should be obtained to determine if this is a factor in this patient's case. Further, TG-lowering drugs, such as a fibrate, nicotinic acid, or omega-3 fatty acids, may also be considered.[1,18] Bile acid sequestrants should be avoided as they may increase TGs. If he experiences epigastric pain or vomiting, it may be prudent to check amylase and lipase levels and proceed with further evaluation for pancreatitis. Once TG levels have been lowered to <500 mg/dL, then attention can be turned to assessment of the lipid panel for ASCVD risk reduction.

saturated fats without a concomitant increase in carbohydrates.[18] For patients with diabetes, glycemic control may help to lower TG concentrations. For very high TGs, the drugs of choice are fibrates, nicotinic acid, or omega-3 fatty acids.[1] An alternative approach to drug therapy for those patients at lower risk for pancreatitis is to intensify statin therapy, which will provide some reduction in TGs. Bile acid sequestrants should be avoided because these agents are known to increase TG concentrations (**Minicase 1**).

In addition to the risk of pancreatitis, extremely high concentrations of TGs—concentrations in excess of 2000 mg/dL (22.6 mmol/L)—may also lead to eruptive cutaneous xanthomas on the elbows, knees, and buttocks. Once TG concentrations are reduced, the xanthomas gradually disappear over the course of one to three months. Hypertriglyceridemia may also manifest as lipemia retinalis (a salmon-pink cast in the vascular bed of the retina). This sign is due to TG particles scattering light in the blood and is seen in the retinal vessels during an eye exam. In patients with lipemia retinalis, TG concentrations may be 4000 mg/dL (45 mmol/L) or greater.[5,40] A concentration this high requires immediate action because it causes hyperviscosity of the blood with the risk of thrombus formation.

Many patients with a high TG concentration lead a sedentary lifestyle and are obese.[1] Patients encountered in clinical practice with elevated TGs often have similar lipid and nonlipid risk factors of metabolic origin termed *metabolic syndrome*. The metabolic syndrome is characterized by abdominal obesity, insulin resistance, hypertension, low HDL, and elevations in TGs. Metabolic syndrome is managed by correcting underlying causes with lifestyle modifications and by treating associated lipid risk factors.

Enzymatic methods for TG measurements are susceptible to interference by glycerol which is normally present in serum.[30,35] Clinically significant increases in glycerol concentrations can also occur in diabetes or in liver disease. However, clinical laboratories provide a standard correction that is adequate for the majority of specimens. An excess of TGs in the blood can lead to errors in other laboratory measurements.

Patients with severe hypertriglyceridemia may have lipemic samples, characterized by a milky appearance.[41] Although this does not affect laboratory TG measurement, it may cause interference in measurement of other laboratory tests such as alanine aminotransferase and aspartate aminotransferase, which depend on spectrophotometric methods for analysis. Most technologists and automated systems can identify lipemic samples and processes can be used to remove lipemia. This produces a clear specimen and eliminates interferences in these assay methods.

Low-Density Lipoprotein Cholesterol

For adults >20 years[1]:
desirable, <100 mg/dL (<2.58 mmol/L);
above desirable, 100–129 mg/dL (2.58–3.33 mmol/L);
borderline high, 130–159 mg/dL (3.36–4.11 mmol/L);
high, 160–189 mg/dL (4.14–4.89 mmol/L);
or very high, ≥190 mg/dL (≥4.91 mmol/L)

Low-density lipoprotein (LDL) cholesterol is ideally measured while the patient is in a fasting state. LDL can be measured directly or estimated indirectly by a method determined by Friedewald.[42] The Friedewald formula subtracts the HDL and VLDL cholesterol from the total plasma cholesterol. The VLDL is estimated to be the plasma TG level divided by five. Using the following formula (all in milligrams per deciliter), LDL may be estimated in patients with a TG concentration <400 mg/dL (<4.52 mmol/L):

$$\text{LDL} = \text{TC} - \text{HDL} - (\text{TGs}/5)$$

If a patient's serum TG concentration exceeds 400 mg/dL (4.52 mmol/L), LDL cholesterol cannot be calculated with this formula. A direct LDL measurement by laboratory would provide an LDL value; however, direct LDL measurement is more expensive. Because treatment of hypertriglyceridemia would take priority in such a patient, most clinicians would treat hypertriglyceridemia first. Once TG values have decreased to <400 mg/dL (<4.52 mmol/L), a standard lipid panel would provide LDL cholesterol data.

One approach to dyslipidemia management is a LDL goal-centered approach. An alternative approach steps away from centering on goals and focuses on therapies to reduce ASCVD risk. The NLA's LDL goal-centered approach begins with assessing presence of clinical ASCVD (e.g., MI, TIA, or peripheral arterial disease) or ASCVD risk factors (e.g., premature family history, current cigarette smoking, and low HDL).[1] This assessment is used to categorize the patient's level of risk for ASCVD. The LDL goal identified for those at low, moderate, or high risk for ASCVD is <100 mg/dL (2.58 mmol/L), whereas the LDL goal for those at very high risk (e.g., clinical ASCVD) is <70 mg/dL (1.81 mmol/L).

ACC/AHA guidelines again require an assessment of the presence of clinical ASCVD but also seek to identify those with very high LDL levels (≥190 mg/dL or 4.91 mmol/L).[2] These two patient groups are identified as those that would benefit from a high-intensity statin. For all other patients (i.e., patients considered for primary prevention therapy and patients with diabetes), an estimation of ASCVD risk is recommended via use of a risk calculator. A patient's ASCVD risk will determine if statin therapy is warranted and if so the intensity of statin therapy (**Minicase 2 and Minicase 3**).

Other guidelines addressing dyslipidemia management in specific patient populations may provide additional information for clinicians. These guidelines also fall into one of the two approaches to dyslipidemia management. For example, the American Association of Clinical Endocrinologists (AACE) recommends a goal-based approach with an LDL goal of <100 mg/dL for patients with diabetes at moderate risk for ASCVD risk, but AACE recommends the intensified goal of <70 mg/dL for patients at high risk.[23] In contrast, the American Diabetes Association recommends an intensity of drug therapy based on risk factors for ASCVD or the presence of clinical ASCVD and does not provide target LDL goals.[43]

Lifestyle modifications are appropriate for all patients who would benefit from LDL lowering. Detailed education should be provided to patients regarding the adoption of a low saturated fat diet that reduces the percent of calories from trans fats and saturated fats such as the Dietary Approaches to Stopping Hypertension (DASH) diet or AHA diet.[19] In certain populations, the DASH dietary approach may reduce LDL by 11 mg/dL. Physical activity should also be encouraged with evidence supporting reductions in LDL of 3–6 mg/dL with aerobic physical activity. Weight loss in patients who are overweight and obese should be encouraged, with an initial weight loss goal of 5–10% of body weight.[1] For patients at elevated ASCVD risk, lifestyle modifications alone should not be recommended. Instead, concurrent drug therapy should be initiated with statins being the preferred therapy.[1,2] Additionally, based on trial evidence demonstrating reductions in ASCVD risk, it is recommended that when statins are used, doses that achieve at least a 30–40% reduction in LDL should be targeted.

High-Density Lipoprotein Cholesterol

For adults >20 years[1]:
low (men), <40 mg/dL (<1.03 mmol/L);
low (women), <50 mg/dL (<1.29 mmol/L)

Based on epidemiological evidence, *high-density lipoprotein (HDL) cholesterol* acts as an antiatherogenic factor and is often termed "good cholesterol."[1] Although a high HDL concentration is associated with cardioprotection, low levels are associated with increased risk of ASCVD. The Framingham Heart Study demonstrated that higher HDL levels are protective against cardiovascular risk even in the setting of elevations in LDL.[44] HDL has several antiatherogenic properties, such as reverse cholesterol transport and antiplatelet activity; however, clinical studies aimed at raising HDL have failed to demonstrate a decrease in cardiovascular risk.[1] Therefore, the mechanism of the association between low HDL and cardiovascular risk is not fully understood. It is possible that low HDL may be a marker of other atherogenic changes in the full lipid profile.

The blood specimen does not need to be drawn after a 12-hour fast. However, a fast is usually recommended because HDL is often ordered in combination with LDL. HDL cholesterol <40 mg/dL (1.03 mmol/L) in men or 50 mg/dL (1.29 mmol/L) in women is considered a risk factor of metabolic syndrome.[1] Most patients with low HDL levels have concomitant elevated TG levels. In these patients, lifestyle therapy or drug therapy to decrease other atherosclerotic particles usually results in a desirable increase in HDL. HDL is negatively correlated with TGs, smoking, and obesity and positively correlated with physical activity and smoking cessation.[44] Women, especially premenopausal women, typically have higher HDL levels than men; this is likely due to the beneficial effects of estrogen.[4] There is no specific goal for raising HDL with drug therapy.[1]

MINICASE 2

Primary Prevention

Jane K., a 30-year-old female, presents to the clinic for a new patient consult. She has not received routine medical care previously but is very motivated to maintain her health. Her past medical history includes seasonal allergic rhinitis but no other chronic conditions. The only medication she takes is daily cetirizine during summer months. She does not follow a specific diet but notes she tries to limit her fast-food intake and eat a variety of fruits and vegetables. She walks at a moderate pace for 30 minutes three times a week. She denies any current tobacco use (quit five years ago after smoking one pack per day for five years). She reports only drinking on special occasions (two to three times per year). She is unable to provide a detailed family history because she has limited contact with her parents. At her office visit, she has a normal physical exam with BP 110/70 mm Hg. The following nonfasting laboratory results were obtained yesterday: TC 290 mg/dL, HDL cholesterol 50 mg/dL, TGs 150 mg/dL, LDL cholesterol 210 mg/dL, and glucose 95 mg/dL. Electrolyte, hematology, liver, renal, and thyroid tests are all normal. Jane K. is 5'6" and weighs 150 lb.

QUESTION: How should the lipid results be interpreted?

DISCUSSION: This patient is asymptomatic and follows a reasonable lifestyle that includes a diet rich in fruits and vegetables, routine aerobic exercise, and tobacco and alcohol avoidance. She has no evident secondary causes of dyslipidemia. She does not have type 2 diabetes mellitus, thyroid, renal, or liver disease, and her antihistamine medication has no effects on the lipid panel.

Ideally, a lipid panel should be drawn in a fasting state; however, recent evidence suggests the variation between fasting and nonfasting results is clinically insignificant if labs are drawn after a standard meal. It is estimated that nonfasting LDL can be up to 10% lower and nonfasting TGs up to 20% higher when compared to fasting levels.[32] There are typically no changes noted in TC and HDL when comparing nonfasting to fasting samples. Considering all of this, her LDL is very high, TC is high, and her HDL and TGs are in normal range. The primary concern is the very high LDL because LDL ≥190 mg/dL highly suggests the presence of a primary lipid disorder. Patients with very high LDL cholesterol are known to be at an increased lifetime risk for ASCVD events due to their lifetime exposure to elevated atherogenic cholesterol.[2] Regardless of which therapeutic guidelines are followed, she would be considered a high-risk patient given her very high LDL. In a goal-based approach, her LDL goal would be <100 mg/dL.[1]

QUESTION: Should any other laboratory tests be ordered to assess her cardiovascular risk? What should be done next?

DISCUSSION: A number of emerging risk factors are under investigation including apo B and hs-CRP. Higher levels of apo B have been correlated with elevated cardiovascular risk; however, apo B levels strongly correlate with non-HDL cholesterol levels, which can easily be calculated from her lipid panel (non-HDL = TC − HDL).

240 mg/dL = 290 mg/dL − 50 mg/dL

Her non-HDL levels are very high and would be considered a primary target of therapy along with LDL if following a goal-based approach to dyslipidemia management.[1] Thus, calculation of non-HDL levels are preferred over the routine measurement of apo B.

Increased hs-CRP levels have been associated with elevated cardiovascular risk. The ACCF/AHA guidelines recommend that it may be reasonable to measure hs-CRP in men ≥50 or women ≥60 years old with LDL <130 mg/dL or those younger but at intermediate risk.[45] Given that this patient would be considered high risk because of her very high LDL and non-HDL levels, there is no additional laboratory test needed to evaluate her cardiovascular risk. Both guidelines would support intensive therapy for this patient and additional information would have little impact on the clinical recommendation.

She should be encouraged to adhere to a low saturated fat diet that emphasizes reduced saturated fat and trans fat, such as the DASH or AHA diet, and to maintain her physical activity. Because she is considered at high risk, high-intensity statin therapy would be recommended by both guidelines. ACC/AHA guidelines recommends high-intensity statins for all adult patients with an LDL ≥190 mg/dL.[2] NLA guidelines recommend an LDL goal <100 mg/dL and a non-HDL goal <130 mg/dL, which most likely will be achievable only with a high-intensity statin regimen (≥50% LDL reduction).[1] Once treatment is started, a repeat lipid panel could be ordered in 4–12 weeks to assess for adherence and % reduction in LDL. She should also be counseled to utilize an effective contraceptive and notify her provider if she plans to become pregnant.

Non-High-Density Lipoprotein Cholesterol

For adults >20 years[1]:
desirable, <130 mg/dL (3.36 mmol/L);
above desirable, 130–159 mg/dL (3.36–4.11 mmol/L);
borderline high, 160–189 mg/dL (4.14–4.89 mmol/L);
high, 190–219 mg/dL (4.91–5.66 mmol/L);
very high, ≥220 mg/dL (5.69 mmol/L)

Non-HDL cholesterol (TC – HDL) provides an estimate of the sum of cholesterol carried by atherogenic particles; those that contain apo B such as LDL, VLDL, chylomicrons and Lp(a).[1] Non-HDL is considered a target of therapy in one clinical guideline because it is a marker of apo B levels.[1] Both non-HDL and apo B are better predictors of ASCVD risk than LDL cholesterol levels. For guidelines that use a goal-based approach, the non-HDL cholesterol target of therapy is set at 30 mg/dL greater than the LDL goal.[1] For example, if a patient has an LDL goal of <100 mg/dL, the non-HDL goal will be set at <130 mg/dL.

The assessment of non-HDL cholesterol does not require a fasting sample, which makes it a convenient screening tool.[1] In a goal-based approach, both the LDL and non-HDL goals are primary targets of therapy. The lifestyle modifications and

MINICASE 3

Secondary Prevention

Gina P., a 68-year-old, 5'4", 200-lb female, presents to the clinic following discharge from the hospital last week. Prior to her hospitalization, her past medical history includes obesity, hypertension treated with hydrochlorothiazide, and tobacco dependence. She does not have a history of diabetes or thyroid disorder. At the hospital, she had indigestion and dizziness but no chest pain. She was diagnosed with an MI and given prescriptions at discharge for metoprolol succinate, lisinopril, hydrochlorothiazide, atorvastatin, and low-dose aspirin daily. She has never taken a cholesterol-lowering medication before, and she has not filled her discharge prescriptions as she is unsure if she needs all of the medications and wants to check with the providers in the clinic who know her well. Since her MI last week, she has quit smoking. Gina P. states she tries to cook low-fat for her immediate family, but she often entertains for extended family and does not follow any dietary restrictions for these gatherings. She exercised very little prior to her MI, but she plans on walking regularly once she regains her energy. Her father died of a heart attack at age 54.

Her vital signs include BP 144/86 mm Hg and HR 64 beats/min. Fasting lipid profile: TC 220 mg/dL, TGs 150 mg/dL, LDL 148 mg/dL, and HDL 42 mg/dL. Fasting glucose is 76 mg/dL; electrolyte, hematology, liver, renal, and thyroid tests are all normal.

QUESTION: Was it appropriate to order a lipid profile? How should the lipid profile be interpreted? Should this patient have her prescription for a lipid-lowering medication filled?

DISCUSSION: All patients with clinical ASCVD, including MI, should have a lipid profile performed even though lipid values in the post-MI period may be falsely lowered. It is unknown whether this patient's lipid panel had been previously measured and if the results were within desired range.

Even before her MI at age 68, she was at risk for clinical ASCVD. At that time she was an obese, female smoker—older than 55 years—with hypertension who lived a sedentary lifestyle. Her father died prematurely of atherosclerotic vessel disease; his age at death (54) meets the criteria for a risk factor (<55 years old). Of her risk factors, all except her age and family history are modifiable risk factors, meaning she has the ability to change her risk by making lifestyle changes.[1]

This patient is obese, which may contribute to increases in TGs and decreases in HDL cholesterol; she has no other evidence of disease-related secondary causes of dyslipidemia (diabetes, hypothyroidism, obstructive liver disease, or renal dysfunction). However, there are potential substance-related or medication-related secondary causes of dyslipidemia in her case. Although short-term use of hydrochlorothiazide may increase LDL cholesterol and TGs, long-term use is not typically associated with lipid changes.[24] She was a smoker but stopped smoking recently. Smoking is associated with increases in TGs and decreases in HDL cholesterol. Her newly prescribed β-blocker may impact the lipid profile by causing decreases in HDL and increases in TGs. However, she should still start therapy with the β-blocker as the benefits of β-blockers in reducing mortality post-MI outweigh the impact on the lipid profile. Further, her LDL cholesterol is elevated and will be the primary target of therapy.

She should initiate therapy with a high-intensity statin (e.g., atorvastatin 40–80 mg, rosuvastatin 20 mg). ACC/AHA recommendations are to initiate a high-intensity statin in all patients with clinical ASCVD, such as an MI.[2] Likewise, a high-intensity statin would be necessary in a goal-based approach to achieve at least a 50% reduction in LDL to meet an established LDL goal of <70 mg/dL and a non-HDL goal of <100 mg/dL.[1] She needs to obtain a 53% reduction in her LDL cholesterol to bring her to the goal of <70 mg/dL. Lifestyle modifications are appropriate for her, including weight loss, increasing physical activity, and a greater emphasis on reducing saturated fat and trans fat intake in the diet. She should be commended for quitting smoking and encouraged to keep her new healthy habit. Therefore, it is reasonable for this patient to fill her prescription for atorvastatin. A lipid panel and LFTs were performed during her hospitalization, and no additional baseline laboratory tests are needed because she has no personal or family history of myopathy warranting a CK level. Regardless of which approach to dyslipidemia management the clinician takes, the lipid profile should be repeated in 4–12 weeks after statin initiation to check for adherence and goal achievement if the clinician is following a goal-based approach.

drug therapy treatment employed for the individual atherogenic particles (i.e., LDL and TGs) will also reduce non-HDL cholesterol.

EMERGING LIPID RISK FACTORS

A number of emerging lipid risk factors for ASCVD are being investigated to varying degrees, including apo B, LDL particle concentration, and Lp(a).[45,46] Apo B is a major component of all atherogenic lipoproteins and higher levels of apo B have been correlated with an increase in ASCVD.[1] However, apo B levels have not demonstrated superiority over non-HDL levels in ASCVD risk prediction; thus, apo B is typically not measured because non-HDL is readily available with a standard lipid profile. The role of LDL particle concentration has also been investigated.[1,45] Even with the same LDL cholesterol level, individuals with a greater concentration of LDL particles have a higher ASCVD risk.[46] The NLA guidelines state that LDL particle concentration may help identify patients still at elevated ASCVD risk despite reaching goal LDL and non-HDL levels.[1] Finally, Lp(a) is an atherogenic lipoprotein that can predict elevated ASCVD risk independent of other atherogenic lipid values.[46] There is concern about the lack of standardization of Lp(a) measurement in clinical laboratories, which explains why some guidelines recommend against routine measurement.[45] No evidence supports treatment targeting any of these elevated emerging lipid risk factors to reduce ASCVD risk. Therefore, the current American College of Cardiology Foundation (ACCF) and AHA Guidelines for Assessment of Cardiovascular Risk in Asymptomatic Adults do not recommend routine advanced lipid testing, such as apo B, Lp(a).[45] Currently measurements of these emerging risk factors are not readily available in clinical practice. Despite this, some experts still support the use of these markers in intermediate risk patients to help identify elevated ASCVD risk.[46]

Certain inflammatory markers have been linked to excess ASCVD risk.[46,47] The ACCF/AHA has recommended measurement of select nonlipid risk factors in certain patient populations.[45] Because inflammation plays a role in the pathophysiology of atherosclerosis, one of the most studied inflammatory markers is high-sensitivity C-reactive protein (hs-CRP). In a meta-analysis, elevated hs-CRP levels were linked to the risk of cardiovascular events, cerebrovascular events, and cardiovascular mortality.[48] In the JUPITER trial, men ≥50 years old or women ≥60 years old with an LDL cholesterol of <130 mg/dL, but with elevated hs-CRP levels of >2 mg/dL, treated with a statin instead of placebo were shown to have a lower rate of major cardiovascular events.[49] Therefore, the ACCF/AHA 2010 Guidelines recommend that it is reasonable to test hs-CRP in men ≥50 or women ≥60 years old with LDL <130 mg/dL who are not on lipid-lowering therapy or those at a younger age but with intermediate risk.[45] Other guidelines also state that elevated hs-CRP levels can aid in assessing ASCVD risk for those patients with an undetermined risk assessment.[1,2,28] The marker is also one factor in the Reynolds Risk Score that is a tool some clinicians may use to help stratify a patient's cardiovascular risk (Minicase 2).[1]

Another nonlipid marker is lipoprotein-associated phospholipase A2 (Lp-PLA2), an enzyme produced by macrophages and lymphocytes that is found on atherogenic lipoproteins.[45] Because levels of Lp-PLA2 are correlated with increased risk of ASCVD, the ACCF/AHA states that it may be reasonable to measure for risk assessment in intermediate risk asymptomatic adults. However, the groups also note that there is currently no information about whether measurement of Lp-PLA2 concentrations will improve clinical outcomes.

POINT-OF-CARE TESTING OPTIONS

In addition to laboratory monitoring, there are home testing kits available to the patient for determination of lipids.[50] Some home testing kits provide results directly to the patient within 30 minutes or less; these kits typically only provide TC results. The clinical applicability of this testing method, which utilizes a fingerstick for obtaining a sample, is limited because TC alone is not enough for an ASCVD risk assessment. Other home testing kits provide results of the full lipid panel. These tests require the patient to apply blood to a card and mail the sample into a laboratory for processing. In addition to home testing methods, there are relatively inexpensive compact devices for *point-of-care testing* (POCT) outside the laboratory that are waived from the Clinical Laboratory Improvement Amendments. These devices enable testing for TC, HDL, and TGs. The LDL is calculated either by the user or the device using the Friedewald formula. Some devices use one cartridge, which can test multiple components of the panel, whereas other devices require a separate cartridge for each individual laboratory test necessitating multiple fingersticks.

One important consideration when evaluating studies of POCT devices is to be aware that some variability may be explained by the fact that different sample types are often compared. For example, a fingerstick provides a sample with capillary blood and a venous draw provides whole blood. Capillary blood samples may provide lower lipid values than venous collection.[30] Nevertheless, the POCT devices are accepted methods for screening for dyslipidemia and are frequently used at health fairs and other screening opportunities. Because guidelines recommend a complete ASCVD risk assessment on all adult patients, it is important that patients still follow up with a provider for a complete cardiovascular risk assessment because lipids are only one component of cardiovascular health. In any POCT setting, quality control, quality assessment, and proper training of personnel should be performed to improve the accuracy of testing.[51] One of the benefits of POCT is that it involves the patient in the laboratory process. These visits become opportunities for the clinician to provide the patient with feedback on progress and reinforce the steps needed to reduce ASCVD risk.

EFFECTS OF HYPOLIPEMIC MEDICATIONS

Clinicians must be aware of how hypolipemic drugs can influence laboratory test results. The ultimate goal of drug therapy is to reduce ASCVD risk, or, in the case of elevated TGs alone, to reduce the risk of pancreatitis. In general, HMG-CoA reductase inhibitors (statins), ezetimibe, niacin, bile acid sequestrants and proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors are considered LDL-lowering drug therapy. Fibrates, niacin, and omega-3 fatty acids are considered drugs for lowering TGs. Specific actions of the drugs are reviewed below.

HMG-CoA Reductase Inhibitors

By inhibiting the enzyme that catalyzes the rate-limiting step in cholesterol synthesis, *HMG-CoA reductase inhibitors*, or *statins*, lower TC and LDL (18–55%, depending on the drug and dose) and may raise HDL as much as 15%.[1] Statins may also decrease TG concentrations (7–30%). The degree of TG-lowering with statins depends on the degree of initial elevation in TGs with more profound reductions in those with hypertriglyceridemia (20–50%). With all statins, maximum effects usually are seen after four to six weeks. A lipid profile can be ordered 4–12 weeks after therapy is initiated to assess efficacy and adherence

Rare adverse effects include serious liver injury and severe muscle injury accompanied by increases in creatine kinase

(CK).[2] Routine monitoring of liver function tests (LFTs) or CK is not necessary. Instead, alanine transaminase (ALT) should be checked prior to statin initiation and if there are potential signs of hepatotoxicity (e.g., unusual fatigue, yellowing of the skin, or dark-colored urine). Transaminase elevations are uncommon, occurring in <1.5% of patients on statins. However, if ALT is greater than three times the upper limit of normal, then the statin should be stopped while potential causes of the increase in transaminases are investigated.[2,52] Although myalgias (e.g., muscle aches or tenderness) are common, severe muscle injury with myonecrosis or elevations in CK are uncommon.[53] CK should be measured at baseline only in those at increased risk for muscle adverse effects such as those with a personal or family history of statin intolerance or muscle disease.[2] Thereafter, CK should be measured if the patient has new or worsened muscle-related complaints (e.g., pain, tenderness).[2,53] If the CK level is greater than three times baseline or the upper limit of normal, then the statin should be stopped for two to four weeks and the clinician should assess for symptom improvement. Numerous trials document the clinical benefits of statins for primary and secondary prevention of ASCVD. The agents are associated with reductions in major cardiovascular events and total mortality and are considered first-line drug therapy.[2]

Ezetimibe

Ezetimibe reduces LDL cholesterol by 13–20% by selectively inhibiting the intestinal absorption of cholesterol.[1] The drug primarily reduces LDL, and because LDL is a component of non-HDL, it also decreases non-HDL levels. However, the drug has minimal impact on TGs (decreasing levels by 5–11%) and HDL levels (increasing levels by 3–5%).[1] Ezetimibe is generally well tolerated. When ezetimibe is coadministered with a statin, ALT should be performed at baseline and when clinically indicated as with statin monotherapy.[2] Existing data support a reduction in cardiovascular outcomes when ezetimibe is coadministered with a statin in high-risk populations; however, statins remain the first-line treatment choice for ASCVD risk reduction.[2]

Bile Acid Sequestrants

Bile acid sequestrants are agents that bind bile acids (e.g., cholestyramine, colesevelam, and colestipol) and lower LDL concentrations by 15–30%, but they may raise TGs, especially if hypertriglyceridemia exists.[1,54] Because these products stay within the enterohepatic circulation, they do not directly cause other systemic effects that may be reflected in laboratory data. Because the bile acid and resin complex is excreted in the feces, the drugs interfere with the absorption of fat-soluble vitamins and some drugs (e.g., digoxin, thyroid supplements, and warfarin) and may affect serum concentrations of these drugs or prothrombin times. Bile acid sequestrants may increase TG levels and should not be used in individuals with TGs >300 mg/dL and should be used with caution in individuals with TG levels of 250–299 mg/dL.[2]

Niacin

Niacin, or nicotinic acid, is a B vitamin that inhibits lipolysis in adipose tissues and decreases free fatty acid production. Therefore, it is used to lower TGs (20–50%).[1] It also tends to raise HDL (15–35%) and lowers LDL (5–25%), therefore providing reductions in non-HDL (8–23%). Niacin may increase serum glucose, uric acid, and LFTs.[55] Baseline and routine monitoring (i.e., every six months) of fasting blood glucose or hemoglobin A1C, uric acid, and LFTs is recommended.[2] The drug should be discontinued if LFTs are greater than two to three times the upper limit of normal. Cutaneous flushing commonly caused by the drug can be minimized by taking aspirin prior to dosing or by taking niacin with a meal. Slow-release or extended-release formulations may minimize flushing. However, hepatotoxicity, detected by an increase in LFTs greater than three times the upper limit of normal, is more often associated with slow-release preparations of niacin.[55] Although earlier studies suggested that niacin was associated with reductions in major coronary events and possibly reductions in total mortality, two recent large studies failed to demonstrate any reduction in cardiovascular events when niacin was added to statin therapy.[2,55] Therefore, at this time its clinical utility is for use in lowering very high TGs.

Fibric Acid Derivatives

Fibric acid derivatives or *fibrates*—gemfibrozil, fenofibrate, and fenofibric acid—activate peroxisome proliferator-activated receptors that regulate lipid metabolism. Fibrates reduce TGs by 20–50% and increase HDL by 10–20%.[1] However, the effect of treatment with a fibrate on LDL is less predictable, ranging from 5% reductions to increases of up to 20%. Increases in LDL may be more likely to be observed in those with very high TGs. Like the statins, the agents are associated with CK elevations; however, routine monitoring is not recommended when fibrates are used as monotherapy.[2] A higher risk of myopathy is observed in patients on combination therapy with fibrates and statins, particularly when gemfibrozil is used.[56] For patients complaining of muscle pain, it is reasonable to check a CK level and follow the same recommendations as outlined in the section on statins. Fibrates are associated with elevations in serum creatinine and the laboratory test should be evaluated at baseline, within three months after initiation and every six months thereafter.[2] Doses should be adjusted for renal dysfunction and the drugs should be discontinued when the estimated glomerular filtration rate is consistently below 30 mL/min. Increases in homocysteine levels have also been reported with fibrates.[56] Because of mixed results on cardiovascular benefits in outcome trials and adverse effects, the agents are primarily used when TG-lowering is needed to decrease the risk of pancreatitis.[56]

Omega-3 Fatty Acids

Prescription and over-the-counter fish oil products predominantly contain the *omega-3 fatty acids* eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).[57] These essential fatty acids inhibit TG and VLDL synthesis in the liver. Omega-3 fatty acids target TGs, with reductions of up to 44% in patients with very high TG levels.[1] The magnitude of the decrease in TGs depends in part on the baseline level, with larger percentage reductions seen in those with higher TG levels.[57] Products that contain both EPA and DHA (e.g., Lovaza, Epanova) may result in increases in LDL in patients with very high TGs, while the EPA-only product (i.e., Vascepa) tends to cause no major changes in LDL.[57,58] Changes in HDL are also variable ranging from a decrease of 5% to an increase of 7%.[58] Combined EPA and DHA products tend to cause an increase in HDL, while EPA-only products cause a decrease.[57] In some patients, increases in ALT levels may be seen, but there are no recommendations for baseline or follow-up monitoring.[2,57] Omega-3 fatty acids may prolong bleeding times.[57] Although bleeding times remain within the normal range and no major bleeding episodes were reported in clinical trials, experts recommend close monitoring of the international normalized ratio when initiating fatty acids for a patient on warfarin therapy. Similar to fibrates, randomized controlled trials of fatty acids have demonstrated inconsistent results and the agents are primarily used when TG-lowering is needed to decrease the risk of pancreatitis.

Proprotein Convertase Subtilisin/Kexin Type 9 Inhibitors

The newest drugs on the market, *proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors,* are approved to target additional LDL lowering in patients on maximally tolerated statin therapy with clinical ASCVD or heterozygous familial hypercholesterolemia.[59,60] These monoclonal antibodies decrease LDL by approximately 60% by increasing hepatocyte LDL receptors, thereby also decreasing non-HDL cholesterol by approximately 50%. Unlike the other medications used to target dyslipidemias, the PCSK9 inhibitors are injectable agents and the patient is instructed on how to self-inject the medication subcutaneously every two weeks or monthly. Following initiation, the lipid panel should be assessed in four to eight weeks. There are no other laboratory monitoring parameters for safety. Trials evaluating the impact of PCSK9 inhibitors on mortality or cardiovascular events are being conducted.

SUMMARY

All adults, age 20 years or older, should have a lipid profile checked once every five years. The lipid profile consists of TC, LDL cholesterol, HDL cholesterol, and TGs. A non-HDL level can be calculated from these lipid components. Assessment of the lipid panel is an important step in identification of patients that may benefit from lifestyle modifications and drug therapy to reduce ASCVD risk. There are two approaches to management of dyslipidemia to mitigate ASCVD risk. One approach is goal-centered; it identifies LDL and non-HDL goals based on risk stratification. An alternative approach steps away from centering on goals and focuses on therapies to reduce ASCVD risk. In the setting of hypertriglyceridemia, assessment of the lipid profile aids the clinician in identifying patients at risk for pancreatitis and assists with diagnostic, prognostic, and therapeutic decisions. For all patients with dyslipidemia, periodic measurement of the lipid profile is recommended to monitor progress and adherence.

LEARNING POINTS

1. ***Who should receive lipid testing?***

   ANSWER: Starting at age 20, all adults should have a lipid profile at a minimum of every five years.[1,28] The lipid profile includes TC, LDL cholesterol, HDL cholesterol, and TGs. Although it is reasonable to measure it once every five years in low-risk individuals, more frequent checks are required for individuals with lipid values in the undesirable range or when there are changes in ASCVD risk factors.[1] Evidence of ASCVD, weight gain, or metabolic syndrome may all be clinical indicators of the need for earlier rescreening. Screening may also be performed more often (e.g., every one to two years) for certain high-risk groups, such as patients with diabetes.[43] Otherwise, follow-up every five years is suggested for a low-risk adult population. Screening is also recommended in all pediatric patients between the ages of 9 and 11.[29]

2. ***Why was no LDL cholesterol measurement reported for a patient in whom a fasting lipid panel was ordered?***

   ANSWER: Typically, no LDL cholesterol value is reported when TGs exceed 400 mg/dL (4.52 mmol/L). Direct measurement of LDL is not commonly performed due to necessary centrifugation and added expense. Instead, LDL cholesterol concentrations can be estimated indirectly by a method determined by Friedewald[42]: LDL = TC − HDL − (TGs/5). If a patient's serum TG concentration exceeds 400 mg/dL (4.5 mmol/L), LDL cholesterol cannot be calculated with this formula. A direct LDL measurement by laboratory would provide an LDL value. However, hypertriglyceridemia may cause variations in direct LDL cholesterol assays as well.[61] Further, because LDL cholesterol is not the primary target of therapy in patients with TGs in excess of 500 mg/dL, the LDL cholesterol value would provide little clinical utility.[1]

3. ***If a lipid panel is drawn in the nonfasting state, are the results clinically usable?***

   ANSWER: The lipid panel is ideally performed under fasting conditions, following a 9–12 hour fast.[30] However, if a patient has any beverage with caloric content or food prior to laboratory measurement, there are no clinically significant changes in TC and HDL cholesterol levels. Because HDL levels are not affected, then the non-HDL levels are similarly unaffected. Historically, clinicians recommended fasting lipid profiles only. However, newer recommendations consider it reasonable to interpret a nonfasting lipid panel because recent evidence suggests that the impact of a low-fat meal on LDL and TG levels is also clinically insignificant.[18,31-33] If TG assessment is the focus of the laboratory or if TGs are >400 mg/dL in a nonfasting sample, then remeasurement of a fasting lipid panel would be required.

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QUICKVIEW | Triglycerides

PARAMETER	DESCRIPTION	COMMENTS
Common reference ranges		
Adults	Normal: <150 mg/dL (1.70 mmol/L)	SI conversion factor: 0.01129 (mmol/L)
	Borderline high: 150–199 mg/dL (1.70–2.25 mmol/L)	
	High: 200–499 mg/dL (2.26–5.64 mmol/L)	
	Very high: ≥500 mg/dL (≥5.65 mmol/L)	
Pediatrics	Acceptable: 0–9 yr: <75 mg/dL (<0.85 mmol/L) 10–19 yr: <90 mg/dL (<1.02 mmol/L)	
	Borderline high: 0–9 yr: 75–99 mg/dL (0.85 −1.12 mmol/L) 10–19 yr: 90–129 mg/dL (1.02–1.46 mmol/L)	
	High: 0–9 yr: ≥100 mg/dL (≥1.13 mmol/L) 10–19 yr: ≥130 mg/dL (≥1.47 mmol/L)	
Critical value	500 mg/dL (5.65 mmol/L)	High risk of pancreatitis
Inherent activity?	Intermediary for other active substances and stored energy in adipose tissue	Needed for formation of other lipids and fatty acids
Location		
Production	Liver and intestines	From ingested food
Storage	Adipose tissue	
Secretion/excretion	None	
Causes of abnormal values		
High	Excess carbohydrate intake	Tables 9-2 and 9-3
	Genetic defects	Associated with obesity, diabetes, and metabolic syndrome
	Drugs	
	Alcohol	
Low	Hypolipidemics	Statins, niacin, fibric acids, omega-3 fatty acids
	Lifestyle modifications	
Signs and symptoms		
High level	Pancreatitis	Increased risk of ASCVD
	Eruptive xanthomas	
	Lipemia retinalis	
Low level	None	
After event, time to…		
Initial elevation	Days to weeks	Single high-fat meal has major effect on TG concentration within 2 hr
Peak values	Days to weeks	Increases with aging
Normalization	Days to weeks	After diet changes or drug treatment is started
Causes of spurious results	Glycerol, recent high-fat meal, alcohol, lipid emulsion	
Additional info	TGs are not the primary target of therapy unless TGs ≥500 mg/dL	

ASCVD = atherosclerotic cardiovascular disease; SI = International System of Units; TG = triglyceride; TGs = triglycerides.

QUICKVIEW | Total Cholesterol

PARAMETER	DESCRIPTION	COMMENTS
Common reference ranges		
Adults	Desirable: <200 mg/dL (<5.17 mmol/L)	SI conversion factor: 0.02586 (mmol/L)
	Borderline high: 200–239 mg/dL (5.17–6.18 mmol/L)	
	High: ≥240 mg/dL (≥6.20 mmol/L)	
Pediatrics	Acceptable: <170 mg/dL (<4.40 mmol/L)	
	Borderline high: 170–199 mg/dL (4.40–5.15 mmol/L)	
	High: ≥200 mg/dL (≥5.17 mmol/L)	
Critical value	Not acutely critical	Depends on risk factors, LDL, TGs, and HDL
Inherent activity?	Intermediary for other active substances	Needed for cell wall, steroid, and bile acid production
Location		
Production	Liver and intestines	Ingested in diet
Storage	Lipoproteins	
Secretion/excretion	Excreted in bile	Also recycled to liver
Causes of abnormal values		
High	Diet high in saturated fats and trans fats	Tables 9-2 and 9-3
	Genetic defects	
	Drugs	
Low	Hyperthyroidism	
	Liver disease	
	Hypolipidemics	
	Lifestyle modifications	Statins, ezetimibe, niacin, fibric acids, bile acid sequestrants, PCSK9 inhibitors
Signs and Symptoms		
High level	Tendon xanthomas	Increased risk of ASCVD
Low level	None	Usually considered sign of good health
After insult, time to…		
Initial elevation	Days to weeks	Single meal has little effect on TC concentration
Peak values	Days to weeks	Can increase with aging; does not change acutely
Normalization	Weeks to months	After diet changes or drugs
Causes of spurious results	Prolonged tourniquet application	Causes venous stasis (increase 5–10%)
Additional Info	TC is not the primary target of therapy	

ASCVD = atherosclerotic cardiovascular disease; HDL = high-density lipoprotein; LDL = low-density lipoprotein; PCSK9 = proprotein convertase subtilisin/kexin type 9; SI = International System of Units; TC = total cholesterol; TGs = triglycerides.

QUICKVIEW | LDL Cholesterol

PARAMETER	DESCRIPTION	COMMENTS
Common reference ranges		
Adults	Desirable: <100 mg/dL (<2.58 mmol/L)	SI conversion factor: 0.02586 (mmol/L)
	Above desirable: 100– 129 mg/dL (2.58–3.33 mmol/L)	
	Borderline high: 130–159 mg/dL (3.36–4.11 mmol/L)	
	High: 160–189 mg/dL (4.14–4.89 mmol/L)	
	Very high: ≥190 mg/dL (≥4.91 mmol/L)	
Pediatrics	Acceptable: <110 mg/dL (<2.84 mmol/L)	
	Borderline high: 110–129 mg/dL (2.84–3.33 mmol/L)	
	High: ≥130 mg/dL (≥3.36 mmol/L)	
Critical Value	Not acutely critical	Depends on risk factors and ASCVD history
Inherent activity?	Intermediary for other active substances	Needed for cell wall, steroid, and bile acid production
Location		
Production	Liver and intestines	Ingested in diet
Storage	Lipoproteins	
Secretion/excretion	Excreted to bile	Also recycled to liver
Causes of abnormal values		
High	Diet high in saturated fats and trans fats, genetic defects, hypothyroidism, nephrotic syndrome	Tables 9-2 and 9-3
Low	Drugs, hyperthyroidism, liver disease, hypolipidemics, lifestyle modifications	Table 9-3
		Statins, ezetimibe, niacin, bile acid sequestrants, PCSK9 inhibitors
Signs and symptoms		
High level	Atherosclerotic vascular disease, tendon xanthomas	Clinical ASCVD
Low level	None	Usually considered sign of good health
After event, time to…		
Initial elevation	Days to weeks	Single meal has little effect on LDL cholesterol concentration; however, ideally measured under fasting state
Peak values	Days to weeks	Can increase with aging; does not change acutely
Normalization	Weeks to months	After diet changes or medications
Causes of spurious results	Acute coronary syndrome	LDL levels decline within few hours of event and may remain low for several weeks
Additional info	LDL may be a target of therapy	
	Indirect methods are typically used to calculate LDL cholesterol with the most common being the Friedewald equation: LDL = TC − HDL − (TGs/5)	
	This equation cannot be used if the specimen is nonfasting, TGs >400 mg/dL, or in patients with familial dysbetalipoproteinemia	

ASCVD = atherosclerotic cardiovascular disease; HDL = high-density lipoprotein; LDL = low-density lipoprotein; PCSK9 = proprotein convertase subtilisin/kexin type 9; SI = International System of Units; TGs = triglycerides.

10

ENDOCRINE DISORDERS

Eva M. Vivian

OBJECTIVES

After completing this chapter, the reader should be able to

- Identify patients who should be screened for diabetes mellitus and determine the diagnostic tests that should be employed
- Recognize and differentiate between the subjective and objective data consistent with a diagnosis of type 1 and type 2 diabetes mellitus and relate this data to the pathogenesis of type 1 and type 2 diabetes mellitus
- Explain the major differences between laboratory values found in diabetic ketoacidosis and in a hyperosmolar hyperglycemic state
- Identify common medications or chemicals that may induce hyperglycemia or hypoglycemia
- Describe the use of glycated hemoglobin, fasting plasma glucose, and oral glucose tolerance tests as diagnostic tools
- Describe the actions of thyroxine, triiodothyronine, and thyroid-stimulating hormone and the feedback mechanisms regulating them
- Recognize the signs and symptoms associated with abnormally high and low concentrations of thyroid hormones
- Given a case description including thyroid function test results, identify the type of thyroid disorder, and describe how tests are used to monitor and adjust related therapy

(continued on page 194)

The endocrine system consists of hormones that serve as regulators, which stimulate or inhibit a biological response to maintain homeostasis within the body. Endocrine disorders often result from a deficiency or an excess of a hormone, leading to an imbalance in the physiological functions of the body. Usually, negative feedback mechanisms regulate hormone concentrations (**Figure 10-1**). Therefore, laboratory assessment of an endocrine disorder is based on the concentrations of a plasma hormone and on the integrity of the feedback mechanism regulating that hormone. In this chapter, the relationship between a hormone (insulin) and a target substrate (glucose) serves as an example of these concepts. Evaluations of the functions of the thyroid and adrenal glands are also described. The relationships between vasopressin (antidiuretic hormone [ADH]) and serum and urine osmolality are used to demonstrate the basis for the water deprivation test in diagnosing diabetes insipidus.

GLUCOSE HOMEOSTASIS

Glucose serves as the fuel for most cellular functions and is necessary to sustain life. Carbohydrates ingested from a meal are metabolized in the body into glucose. Glucose is absorbed from the gastrointestinal (GI) tract into the bloodstream where it is utilized in skeletal muscle and brain for energy. Glucose is also stored in the liver in the form of glycogen (glycogenesis) and is converted in adipose tissue to fats and triglycerides (lipogenesis). Insulin, which is produced, stored, and released from β cells of the pancreas, facilitates these anabolic processes. The liver, skeletal muscle, brain, and adipose tissue are the main tissues affected by insulin. To induce glucose uptake, insulin must bind to specific cell-surface receptors. Most secreted insulin is taken up by the liver, while the remainder is metabolized by the kidneys. About 80% of glucose uptake is independent of insulin. These insulin-independent cells include nerve tissue, red blood cells (RBCs), mucosal cells of the GI tract, and exercising skeletal muscle.[1,2]

In the fasting state, insulin levels decrease, resulting in an increase in glycogen breakdown by the liver (glycogenolysis) and an increase in the conversion of free fatty acids to ketone bodies (lipolysis).[1,2] When glucose concentrations fall below 70 mg/dL, an event known as *hypoglycemia* occurs resulting in the release of glucagon by the pancreatic α cell. Glucagon stimulates the formation of glucose in the liver (gluconeogenesis) and glycogenolysis. Glucagon also facilitates the breakdown of stored triglycerides in adipose tissue into fatty acids (lipolysis), which can be used for energy in the liver and skeletal muscle. In addition to glucagon secretion, hypoglycemia leads to secretion of counter regulatory hormones such as epinephrine, cortisol, and growth hormone. Glucagon and, to a lesser degree, epinephrine promote an immediate breakdown of glycogen and the synthesis of glucose by the liver. Cortisol increases glucose levels by stimulating gluconeogenesis. Growth hormone inhibits the uptake of glucose by tissues when glucose levels fall below 70 mg/dL.[3,4]

Other hormones, such as amylin and incretin, affect glucose concentrations. Discovered in 1987, amylin, a β-cell hormone is cosecreted with insulin at a molar ratio of 1:20–50 in response to a glucose challenge. Amylin is a neuroendocrine hormone that complements the actions of insulin by restraining the vagus nerve-mediated rate of gastric emptying, thereby slowing intestinal carbohydrate absorption and

OBJECTIVES

- **Describe the relationship between urine osmolality, serum osmolality, and antidiuretic hormone as it relates to diabetes insipidus**
- **Describe the laboratory tests used to diagnose Addison disease and Cushing syndrome**

resulting in lower postprandial glucose (PPG) levels. This delay in gastric emptying has been found to be the same in patients with type 1 and type 2 diabetes mellitus (DM) that were without complications. Amylin also suppresses hepatic glucose output by inhibiting glucagon after ingestion of a meal.[5-8] In animal studies, amylin was found to induce postprandial satiety in direct proportion to food intake.[6] The administration of amylin has been reported to decrease food intake, thereby resulting in weight loss in patients with type 2 DM.[9,10]

Recent studies have shown that β-cell response is greater after food ingestion or when glucose is given orally versus after intravenous (IV) glucose infusion. This difference in insulin secretion has been termed the *incretin effect*, which implies that food ingestion causes the release of specific gut hormones known as *incretins* that enhance insulin secretion beyond the release caused by the rise in glucose secondary to absorption of digested nutrients.[11,12] Studies in humans and animals have shown that the incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) stimulate insulin release when glucose levels are elevated.[12-14]

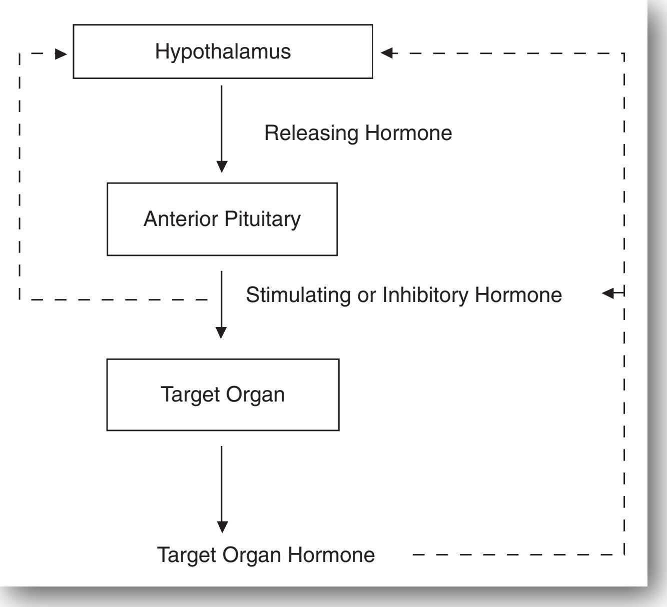


FIGURE 10-1. **The hypothalamus may secrete a releasing hormone in response to low levels of stimulating, inhibitory, or target organ hormone. This releasing hormone causes the release of a stimulating or inhibitory hormone that in turn controls the release of target organ hormone.**

After food is ingested, GIP is released from K cells in the proximal gut (duodenum), and GLP-1 is released from L cells in the distal gut (ileum and colon). Under normal circumstances, dipeptidyl peptidase 4 (DPP-4) rapidly degrades these incretins to their inactive forms after their release into the circulation. As a result, the plasma half-life of GIP and GLP-1 is less than five minutes.[12] GLP-1 and GIP stimulate insulin response in pancreatic β cells, and GLP-1 (but not GIP) also suppresses glucagon production in pancreatic α cells when the glucose level is elevated. The subsequent increase in glucose uptake in muscles and reduced glucose output from the liver help maintain *glucose homeostasis*. Thus, the incretins GLP-1 and GIP are important glucoregulatory hormones that positively affect glucose homeostasis by physiologically helping to regulate insulin in a glucose-dependent manner.[12,15]

The kidney contributes to glucose homeostasis primarily by the reabsorption and return of glucose to the circulation. Glucose is freely filtered by the glomerulus, and in healthy individuals approximately 180 g of glucose are filtered daily, and almost all of this is reabsorbed by the proximal tubule. Glucose reabsorption by the kidney is mediated by a class of specific glucose transport proteins, the sodium–glucose cotransporters (SGLTs). One member of this family, SGLT2, is responsible for the majority of renal glucose reabsorption and is located on the luminal side of cells in the initial part of the nephron, the early proximal convoluted tubule. Another member of this family, SGLT1, is expressed mainly in the intestine, but it is also present in skeletal muscle, heart, and based on animal studies in the late proximal tubule where it accounts for additional glucose reabsorption from the glomerular filtrate. Glucose is taken up into the cell by SGLTs and exits across the basolateral membrane into the interstitium by facilitative diffusion via the facilitative glucose transporters GLUT2 and GLUT1.[16]

In individuals without diabetes, once plasma glucose concentrations exceed approximately 180 mg/dL (the renal threshold), renal glucose reabsorption is saturated and glucose starts to appear in the urine. In hyperglycemic individuals, the renal threshold may be exceeded, and large amounts of glucose may be excreted in the urine. However, the kidneys continue to reabsorb glucose, and in patients with type 2 DM, the renal capacity to reabsorb glucose may be increased, which further contributes to hyperglycemia.[17]

In summary, glucose concentrations are affected by any factor that can influence glucose production or utilization, glucose absorption from the GI tract, glycogen catabolism, or insulin production or secretion. Fasting suppresses the rate of insulin secretion, and feasting generally increases insulin secretion. Increased insulin secretion lowers serum glucose concentrations, while decreased secretion raises glucose concentrations.[1-4]

DIABETES MELLITUS

The three most commonly encountered types of diabetes mellitus (DM) include the following:

1. Type 1 DM, formally known as *insulin-dependent DM* (IDDM)

2. Type 2 DM, formerly known as *adult onset or noninsulin dependent DM* (NIDDM)[18]
3. Gestational DM

Type 1 DM is characterized by a lack of endogenous insulin, predisposition to ketoacidosis, and an abrupt onset. Some patients present with ketoacidosis after experiencing polyuria, polyphagia, and polydipsia for several days. Typically, this type of DM is diagnosed in children and adolescents but may also occur at a later age. In contrast, patients with type 2 DM are not normally dependent on exogenous insulin to sustain life and are not ketosis prone, but they are usually obese and are more than 40 years old. There is an alarming increase in the number of children and adolescents diagnosed with type 2 DM. Although there is a genetic predisposition to the development of type 2 DM, environmental factors such as high-fat diets and sedentary lifestyles contribute to the disorder. Type 2 DM patients are both insulin deficient and insulin resistant (**Table 10-1**).[1,2]

Many type 2 DM patients are asymptomatic so diagnosis often depends on laboratory studies. Concentrations of ketone bodies in the blood and urine are typically low or absent, even in the presence of hyperglycemia. This finding is common because the lack of insulin is not severe enough to lead to abnormalities in lipolysis and significant ketosis or acidosis.

Because of the chronicity of asymptomatic type 2 DM, many patients with type 2 DM present with evidence of microvascular complications (neuropathy, nephropathy, and retinopathy) and macrovascular complications (coronary artery, cerebral vascular, and peripheral arterial disease) at the time of diagnosis. Type 2 DM is often discovered incidentally during glucose screening sponsored by hospitals and other healthcare institutions.[1,2]

Gestational DM, a third type of glucose intolerance, develops during the third trimester of pregnancy. Patients with gestational DM have a 30–50% chance of developing type 2 DM.[19] A woman with diabetes who becomes pregnant, or a woman diagnosed with diabetes early in her pregnancy, are not included in this category.

Pathophysiology

Type 1 DM usually develops in childhood or early adulthood and accounts for up to 10% of all patients with diabetes. Patients are usually thin, have an absolute lack of insulin, and require exogenous insulin to prevent diabetic ketoacidosis (DKA) and sustain life. Genetics as well as environmental and immune factors are the major factors thought to cause type 1 DM.[20]

Over 90% of all type 1 DM patients have a combination of human leukocyte antigen (HLA-DQ) coded genes, which increase the risk of developing type 1 DM. Type 1 DM may result from a trigger, which could be environmental or viral. Viral infections may stimulate monocytes and macrophages that activate T cells, which attack β cells, thereby decreasing insulin production. Bovine serum albumin has also been identified as an environmental trigger. Therefore, it is believed that exposure to cow's milk may increase the risk of developing type 1 DM in patients with a genetic predisposition. An immunologic attack against insulin may also occur. The combination of an autoimmune attack on β cells and on circulating insulin results in insulin insufficiency.[20-22]

Type 2 DM involves multiple organ dysfunction that results in insulin resistance and insulin deficiency. During the early stages of type 2 DM, the ability of insulin to facilitate the diffusion of glucose into the cell is impaired. Defects in insulin receptor function, the insulin receptor-signal transduction pathway, glucose transport and phosphorylation, and glycogen synthesis and oxidation contribute to muscle insulin resistance. The pancreas compensates for this deficiency by secreting larger amounts of insulin. Patients remain euglycemic until the pancreatic β cells are no longer able to compensate for the insulin resistance. Hence, insulin is unable to suppress hepatic glucose production and hyperglycemia results.[1,22-24]

Recently, other causes for the excessive postprandial increase in plasma glucose levels have been identified. Because amylin is cosecreted with insulin, there is an absolute deficiency of this hormone in patients with type 1 DM. In patients with type 2

TABLE 10-1. General Characteristics of Type 1 and Type 2 Diabetes Mellitus

CHARACTERISTICS	TYPE 1	TYPE 2
Age of onset	Childhood or adolescence	>40 yr old
Rapidity of onset	Abrupt	Gradual
Family studies	Increased prevalence of type 1 DM	Increased prevalence of type 2 DM
Body weight	Usually thin and undernourished	Obesity is common
Islet cell antibodies and pancreatic cell-mediated immunity	Yes	No
Ketosis	Common	Uncommon; if present associated with severe stress or infection
Insulin	Markedly diminished early in disease or totally absent	Levels may be low, normal, or high (indicating insulin resistance)
Symptoms	Polyuria, polydipsia, polyphagia, weight loss	May be asymptomatic; polyuria, polydipsia, polyphagia may be present

DM = diabetes mellitus; yr = years old.

DM, amylin levels decrease progressively. Whether due to the destruction of pancreatic β cells in patients with type 1 DM or the progressive exhaustion of β-cell function in patients with type 2 DM, amylin deficiency is now recognized as a contributor to the development of DM.[12-15]

The incretin effect is abnormal in those with type 2 DM mellitus. The diminished incretin effect observed in patients with type 2 DM may be due to reduced responsiveness of pancreatic β cells to GLP-1 and GIP or to impaired secretion of the relevant incretin hormone.[13]

Secondary Causes

DM may be the result of other pancreatic and hormonal diseases, medications, and abnormalities of the insulin receptor.[13] Pancreatic cell destruction may be related to diseases, such as cystic fibrosis, or autoimmune conditions. It can be related to medications (e.g., L-asparaginase, streptozocin, and pentamidine). Similarly, hyperglycemia may result from hormonal disease in which concentrations of circulating catecholamines and glucocorticoids are increased (e.g., catecholamine-secreting pheochromocytoma and Cushing syndrome, respectively).[13]

Various medications or chemicals may induce hyperglycemia and glucose intolerance. β-adrenergic antagonists may decrease or impair insulin secretion in patients with diabetes or may decrease glycogenolysis and gluconeogenesis. Diazoxide and phenytoin may decrease insulin secretion resulting in increased glucose levels. High doses of thiazides and loop diuretics may cause hyperglycemia by an unknown mechanism.[24] It should be noted that potassium-sparing diuretics have little or no effect on glucose levels. Estrogen products influence glucose tolerance to varying degrees, depending on the formulation. In one study, women taking combination oral contraceptives for at least three months had plasma glucose concentrations 43–61% higher than controls.[25] Monophasic and triphasic combination products diminished glucose tolerance, whereas progestin-only products did not. Insulin resistance was the proposed mechanism, and fasting plasma glucose (FPG) concentrations were not adversely affected. **Table 10-2** lists medications with the potential to cause hyperglycemia.

TABLE 10-2. Medications and Chemicals That May Cause Hyperglycemia[11,25,26]

Diuretics	
Chlorthalidone	Metolazone
Loop diuretics[a]	Thiazides
Steroids/hormones	
Estrogens	Oral contraceptives[c]
Glucocorticoids	Thyroid hormones
Antihypertensives	
Calcium antagonists[b]	Diazoxide
Clonidine[b]	β-blockers
Miscellaneous drugs	
Amiodarone[b]	Epinephrine[d]
L-asparaginase	Lithium[b]
Phenothiazines[b]	
Nicotinic acid	
Protease inhibitors	
Ritonavir	Tipranavir
Indinavir	Atazanavir
Saquinavir	Fosamprenavir
Nelfinavir	Amprenavir
Darunavir	Pentamidine[e]
Nucleoside reverse transcriptase inhibitors	
Didanosine	Zidovudine
Emtricitabine	Phenytoin
Lamivudine	Cyclosporine
Stavudine	Tacrolimus
Tenofovir	Streptozocin
Atypical antipsychotics	
Quetiapine	Olanzapine
Clozapine	Risperidone

[a] Loop diuretics are furosemide, bumetanide, torsemide, and ethacrynic acid. Torsemide has less effect on glucose concentrations.[24]

[b] Clinical significance is less clear.

[c] Progestin-only products do not affect glucose tolerance.

[d] Oral sympathomimetics, such as those found in decongestants, are unlikely to be more of a cause of increased glucose than the "stress" from the illness for which they are used.

[e] After initial hypoglycemia, which occurs in about 4–14 (average 11) days.

Diagnostic Laboratory Tests

C-Peptide

Fasting range: 0.78–1.89 ng/mL (0.26–0.63 nmol/L)

Range one hour after a glucose load: 5–12 ng/mL (1.66–3.97 nmol/L) during a glucose tolerance test

C-peptide levels are used to evaluate residual β-cell function. A C-peptide test distinguishes between type 1 and type 2 DM; identifies the cause of hypoglycemia; and checks that a pancreatic tumor (insulinoma) was completely removed. Insulin is synthesized in the β cells of the islets of Langerhans as the precursor, proinsulin, is cleaved to form C-peptide and insulin, which are both secreted in equimolar amounts into the portal circulation. By measuring the levels of C-peptide, the level of insulin can also be calculated. High levels of C-peptide generally indicate high levels of endogenous insulin production. This may be a response to high levels of blood glucose caused by glucose intake and insulin resistance. Also, high levels of C-peptide also are seen with insulinomas (insulin-producing tumors) and may be seen with hypokalemia, pregnancy, Cushing syndrome, and renal failure. Low levels of C-peptide are associated with low levels of insulin production. This can occur when insufficient insulin is being produced by the β cells (production is suppressed by exogenous insulin) or with suppression tests that involve substances such as somatostatin.[26]

A C-peptide test can be done when DM has just been found and it is not clear whether type 1 or type 2 DM is present. A patient with type 1 DM has a low level of insulin and C-peptide. A patient with type 2 DM has a normal or high level of C-peptide. When a patient has newly diagnosed type 1 or type 2 DM, C-peptide can be used to help determine how much insulin the patient's pancreas is still producing. With type 2 DM, the test may be ordered to monitor the status of β-cell function and insulin production over time and to determine if insulin injections may be required. Also, a C-peptide test can help identify the cause of hypoglycemia, such as excessive use of medicine to treat diabetes or a noncancerous growth (tumor) in the pancreas (insulinoma). Because synthetic insulin does not have C-peptide, a patient with hypoglycemia from taking too much insulin will have a low C-peptide level. An insulinoma causes the pancreas to release too much insulin, which causes hypoglycemia. Such a patient will have a high level of C-peptide in the blood.[26]

Even though they are produced at the same rate, C-peptide and insulin leave the body by different routes. Insulin is processed and eliminated mostly by the liver, while C-peptide is removed by the kidneys. The half-life of C-peptide is about 30 minutes as compared to the half-life of insulin, which is five minutes. Thus, there will be usually about five times as much C-peptide in the bloodstream as insulin.[26]

Diabetes-Related Autoantibody Testing

Diabetes-related (islet) autoantibody testing is used to distinguish between autoimmune type 1 DM and type 2 DM, which allows for early initiation of the most appropriate treatment and may minimize disease complications. The four most common autoantibody tests are islet cell cytoplasmic autoantibodies (ICA), glutamic acid decarboxylase autoantibodies (GADA), insulinoma-associated-2 autoantibodies (IA-2A), and insulin autoantibodies (IAA). Of these, ICA and GADA, which are autoantibodies directed against islet cell proteins or β-cell antigen, are present in 70–80% of adult patients with type 1 DM. IA-2A autoantibodies are present in approximately 60% of adult patients with type 1 DM. The majority of people, 95% or more, with new-onset type 1 DM will have at least one islet autoantibody.[26] Some people who have type 1 DM will never develop detectable amounts of islet autoantibodies, but this is rare.

The autoantibodies seen in children are often different than those seen in adults. IAA is usually the first marker to appear in young children. As the disease evolves, IAA may disappear and ICA, GADA, and IA-2A become more important. Approximately 50% of children with new-onset type 1 DM will be IAA positive.

A combination of these autoantibodies may be ordered when a person is newly diagnosed with diabetes and the healthcare provider wants to distinguish between type 1 and type 2 DM. In addition, these tests may be used when the diagnosis is unclear in persons with diabetes who have been diagnosed as type 2 DM, but who have great difficulty in controlling their glucose levels with oral medications. If ICA, GADA, and IA-2A are present in a person with symptoms of DM, the diagnosis of type 1 DM is confirmed. Likewise, if IAA is present in a child with DM who is not insulin-treated, type 1 DM is the cause. If no diabetes-related autoantibodies are present, then it is unlikely that the diabetes is type 1 DM.

Laboratory Tests to Assess Glucose Control

The two most common methods used for evaluating glucose homeostasis are the FPG and glycated hemoglobin (A1c). The oral glucose tolerance test (OGTT) is mainly used to assess equivocal results from these two tests. A1c and fructosamine tests are used to monitor long-term and medium-term glucose control, respectively. Urine glucose monitoring in patients with DM has been replaced by fingerstick blood glucose tests. Therefore, discussion of this test is relatively brief.

With all the blood tests, proper collection and storage of the sample and performance of the procedure are important. Improper collection and storage of samples for glucose determinations can lead to false results and interpretations. After collection, RBCs and white blood cells continue to metabolize glucose in the sample tube. This process occurs unless (1) the RBCs can be separated from the serum using serum separator tubes, or (2) metabolism is inhibited using sodium fluoride–containing (gray-top) tubes or refrigeration of the specimen. Without such precautions, the glucose concentration drops by 5–10 mg/dL (0.3–0.6 mmol/L) per hour, and the measured glucose level will not reflect the patient's FPG at collection time. In vitro, metabolic loss of glucose is hastened in samples of patients with leukocytosis or leukemia.[27]

Fasting Plasma Glucose and Two-Hour Postprandial Glucose

The categories of *fasting plasma glucose* (FPG) values are as follows:

- FPG <100 mg/dL (5.6 mmol/L) represents normal fasting glucose
- FPG ≥100 (5.6 mmol/L) and <126 mg/dL (7 mmol/L) represents *prediabetes* (previously termed *impaired fasting glucose*)
- FPG ≥126 mg/dL (7 mmol/L) represents provisional diagnosis of diabetes (the diagnosis must be confirmed as described in **Table 10-3**)[18]

An FPG concentration is the best indicator of glucose homeostasis. This test measures the ability of endogenous or exogenous insulin to prevent fasting hyperglycemia by regulating glucose anabolism and catabolism. FPG may be used to monitor therapy in patients being treated for glucose abnormalities. For this test, the patient maintains his or her usual diet, and the assay is performed on awakening (before breakfast). This timing usually allows for an eight-hour fast. An FPG >126 mg/dL (>7 mmol/L), found on at least two occasions, is diagnostic for DM (Table 10-3).

TABLE 10-3. Diagnosis of Diabetes Mellitus Based on Fasting Plasma Glucose Concentration, Oral Glucose Tolerance Test, or Glycosylated Hemoglobin[18]

		VENOUS PLASMA GLUCOSE[a] (mg/dL)		
LEVEL OF GLUCOSE TOLERANCE	**FPG**	**OGTT VALUE (30, 60, or 90 min)**	**OGTT VALUE (2 hr)**	**A1c**
"Normal"	<100	<200	<140	<5.7%
Prediabetes	100–125	>200	140–199	5.7–6.4%
DM	≥126	>200	>200	≥6.5%
Gestational DM	>92	>180 (1 hr)	>153	

A1c = glycosylated hemoglobin; DM = diabetes mellitus; FPG = fasting plasma glucose; hr = hour/s; min = minutes; OGTT = oral glucose tolerance test.
[a]Multiply number by 0.056 to convert glucose to International System (SI) units (mmol/L).

Testing in asymptomatic people should be considered in adults of any age who are overweight or obese (body mass index [BMI] ≥25 kg/m^2) with one or more risk factors[18]:

- Physical inactivity
- A1c ≥5.7%, impaired glucose tolerance or elevated fasting glucose on a previous testing
- First-degree relative with diabetes
- Polycystic ovary syndrome
- Vascular disease, hypertension, or dyslipidemia
- High-risk ethnic groups (e.g., high-risk ethnic group: Hispanic, African American, Native American, South or East Asian, or of Pacific Island descent)
- Women with a prior history of gestational DM

Individuals without these risk factors should be screened no later than 45 years of age. The American College of Endocrinology recommends that individuals from high-risk groups, aged >30 years, be screened for DM every three years.[29]

Oral Glucose Tolerance Test

The categories for the *oral glucose tolerance test* (OGTT) are as follows:

- Two-hour postload glucose (PG), <140 mg/dL (7.8 mmol/L) represents normal glucose tolerance
- Two-hour PG 140–199 mg/dL (7.8–11 mmol/L) represents prediabetes (previously termed *impaired glucose tolerance*
- Two-hour PG ≥200 mg/dL (≥11.1 mmol/L) represents provisional diagnosis of diabetes (the diagnosis must be confirmed as described in Table 10-3)

The OGTT is used to assess patients who have signs and symptoms of DM but whose FPG is normal or suggests prediabetes (<126 mg/dL or <7 mmol/L). The OGTT measures both the ability of the pancreas to secrete insulin following a glucose load and the body's response to insulin. Interpretation of the test is based on the plasma glucose concentrations drawn before and during the exam. This exam may also be used in diagnosing DM with onset during pregnancy if the disease threatens the health of the mother and fetus. The OGTT is not required for patients who have FPG levels >126 mg/dL (7 mmol/L) on at least two separate occasions as such values confirm the diagnosis of DM.[28]

The OGTT is performed by giving a standard 75-g dose of an oral glucose solution over five minutes following an overnight fast. The pediatric dose is 1.75 g/kg up to a maximum of 75 g. Blood samples commonly are drawn before the glucose load, and at 30, 60, 90, and 120 minutes after the glucose load. The samples should be collected into tubes containing sodium fluoride unless the assay will be performed immediately.[28]

If the patient vomits the test dose, the exam is invalid and must be repeated. The OGTT is diagnostic for DM if the two-hour plasma glucose is at least 200 mg/dL (11.1 mmol/L). The patient is considered to have prediabetes if the two-hour concentration is 140–199 mg/dL (7.8–11 mmol/L).

Pregnant women with risk factors—overweight or obese with a BMI >25; family history of type 2 DM; hypertension; hyperlipidemia; and high-risk ethnic group: Hispanic, African American, Native American, South or East Asian, or of Pacific Island descent—should be screened at the first prenatal visit using standard diagnostic criteria. Pregnant women not previously known to have DM or risk factors can be screened for gestational DM at 24–28 weeks gestation. A screening test is performed that measures plasma glucose fasting and plasma glucose at one and two hours after a 75-g oral glucose load. The diagnosis of gestational DM can be made when any of the following plasma glucose values are exceeded:

- FPG ≥92 mg/dL (5.1 mmol/L)
- OGTT value at one hour ≥180 mg/dL (10 mmol/L)
- OGTT value at two hours ≥153 mg/dL[18] (8.5 mmol/L)

The OGTT should not be performed on individuals who are chronically malnourished, consume inadequate carbohydrates (<150 g/day), or are bedridden. Alcohol consumption and medications that cause hyperglycemia (Table 10-2) should be stopped, if possible, three days prior to the OGTT. Coffee and smoking are not permitted during the test.[28]

Glycated Hemoglobin

Normal range: 4–5.6%

Glycated hemoglobin (A1c), also known as *glycosylated A1c,* is a component of the hemoglobin molecule. During the 120-day lifespan of an RBC, glucose is irreversibly bound to the hemoglobin moieties in proportion to the average serum glucose. The process is called *glycosylation.* Measurement of A1c is, therefore, indicative of glucose control during the preceding two to three months. The entire hemoglobin A1 molecule—composed of A1a, A1b, and A1c—is not used because subfractions A1a and A1b are more susceptible to nonglucose adducts in the blood of patients with opiate addiction, lead poisoning, uremia, and alcoholism.[30,31] Because the test measures a component of hemoglobin, the specimen analyzed is RBC, and not serum or plasma.

Results are not affected by daily fluctuations in the blood glucose concentration, and a fasting sample is not required. Results can reflect overall patient compliance to various treatment regimens. With most assays, 95% of a normal individual's hemoglobin is 4–6% glycated. An A1c ≥7% suggests poor glucose control. Patients with persistent hyperglycemia may have an A1c up to 20%.[30]

For years, the A1c has been used to monitor glucose control in people already diagnosed with DM. Initially it was not recommended for diagnosis because the test variability from laboratory to laboratory was too great for a diagnostic test. The A1c cut point of ≥6.5% identifies one-third fewer cases of undiagnosed DM than a fasting glucose cut point of ≥126 mg/dL. However, the lower sensitivity of the test at the cut point is offset by the test's greater practicality, and wider use of this more convenient test may result in an increase in the number of diagnoses made.[18]

A few situations confound interpretation of test results. False elevations in A1c may be noted with uremia, chronic alcohol intake, and hypertriglyceridemia.[31] Patients who have diseases with chronic or episodic hemolysis (e.g., sickle cell disease and thalassemia) generally have spuriously low A1c concentrations caused by the predominance of young RBCs (which carry less A1c) in the circulation. In splenectomized patients and those with polycythemia, A1c is increased. If these disorders are stable, the test still can be used, but values must be compared with the patient's previous results rather than published normal values. Both falsely elevated and falsely lowered measurements of A1c may also occur during pregnancy. Therefore, A1c should not be used to screen for gestational DM.[32-34]

Affinity chromatography and colorimetric assay methods measure total A1c including subfractions A1a and A1b. Ion-exchange chromatography and high-performance liquid chromatography only measure the subfraction. The Cholestech GDX and the Metrika A1cNow are portable analyzers, which provide A1c results within five to eight minutes.[33] The American Diabetes Association (ADA) recommends A1c testing one to two times a year for patients with good glycemic control and quarterly in patients with poor control or whose therapy has changed.[18]

Fructosamine

Normal range: 51 mg/L (<285 μmol/L)

Fructosamine is a general term that is applied to any glycosylated protein. Unlike the A1c test, only glycosylated proteins in the serum or plasma (e.g., albumin)—not erythrocytes—are measured. In nondiabetics, the unstable complex dissociates into glucose and protein. Therefore, only small quantities of fructosamine circulate. In patients with DM, higher glucose concentrations favor the generation of more stable glycation, and higher concentrations of fructosamine are found.

Fructosamine has no known inherent toxicological activity but can be used as a marker of medium-term glucose control. Fructosamine correlates with glucose control over two to three weeks based on the half-lives of albumin (14–20 days) and other serum proteins (2.5–23 days). As a result, high-fructosamine concentrations may alert caregivers to deteriorating glycemic control earlier than increases in A1c.

Falsely elevated results may occur for the following:

- Serum (not whole blood) hemoglobin concentrations are >100 mg/dL (normally <15 mg/dL)
- Serum bilirubin is >4 mg/dL
- Serum ascorbic acid is >5 mg/dL[36]

Methyldopa and calcium dobesilate (the latter is used outside the United States to minimize myocardial damage after an acute infarction) may also cause falsely elevated results. Serum fructosamine concentrations are lower in obese patients with DM as compared to lean patients with DM.[36] Falsely low fructosamine levels can be observed in patients with low serum protein or albumin levels. Some clinicians advocate the use of fructosamine concentrations as a monitoring tool for short-term changes in glycemic control (e.g., gestational DM). However, more clinical studies are required to determine if this test provides useful clinical information.[37,38]

Urine Glucose

Normal range: negative

Glucose "spills" into the urine when the serum glucose concentration exceeds the renal threshold for glucose reabsorption (normally 180 mg/dL). However, a poor correlation exists between *urine glucose* and concurrent serum glucose concentrations. This poor correlation occurs because urine is "produced" hours before it is tested, unless the inconvenient double-void method (urine is collected 30 minutes after emptying of the bladder) is used. Furthermore, the renal threshold varies among patients and tends to increase in diabetes over time, especially if renal function is declining. Urine testing gradually has been replaced by convenient fingerstick blood sugar testing, Urine glucose testing should be recommended only if the patient is unable or unwilling to perform blood glucose monitoring.[39]

The presence and amount of glucose in the urine can be determined by two different techniques. Commercial products that use copper-reducing methods (e.g., Clinitest) provide the most quantitative estimate of the degree of glycosuria. Therefore, this method is preferred for patients who spill large quantities of glucose into the urine. The two-drop method can detect higher concentrations of glucose up to 5% as compared to the five-drop method, which can quantitate up to only 2%. Generally, copper-reducing products have poor specificity for glucose.

Contrary to popular belief, isoniazid, methyldopa, and ascorbic acid do not interfere with Clinitest. The literature is unclear on whether chloral hydrate, nitrofurantoin, probenecid, and nalidixic acid interfere with this method. Patients should be aware that a "pass-through" phenomenon occurs with this test when more than 2% glucose is in the urine. During the test, a fleeting orange color may appear at the climax of the reaction and fade to a greenish-brown when the reaction is complete. The latter color (0.75–1% glucose) may be incorrectly used to assess glucose levels, and the glucose concentration actually may be underestimated.

The second method of urine glucose testing is specific for glucose and provides a more qualitative assessment of it in the urine. Commercial products of this type (e.g., Tes-Tape, Clinistix, Diastix, and Chemstrip) consist of plastic dipsticks with paper pads that have been impregnated with glucose oxidase. These tests are sensitive to 0.1% glucose (100 mg/dL). Substances that may cause false-negative results with this type of test include ascorbic acid (high dose), salicylates (high dose), and levodopa. Interference also has been reported with phenazopyridine and radiographic contrast media, but the interference is unpredictable.[40-42]

Self-Monitoring Tests of Blood Glucose

Blood glucose meters and *reagent test strips* are commercially available so that patients may perform blood glucose monitoring at home. These systems are also used frequently in hospitals, where healthcare providers rely on quick results for determining insulin requirements. The meters currently marketed are lightweight, relatively inexpensive, accurate, and user-friendly.[43]

The first generation of self-monitoring blood glucose (SMBG) meters relied on a photometric analysis that was based on a dye-related reaction. This method, also termed *reflectance photometry, light reflectance,* or *enzyme photometric,* involves a chemical reaction between capillary blood and a chemical on the strip that produces a change in color. The amount of color reflected from the strip is measured photometrically. The reflected color is directly related to the amount of glucose in the blood. The darker color the test strip, the higher the glucose concentration. The disadvantages of this method are that the test strip has to be developed after a precise interval (after the blood is washed away), a large sample size of blood (>12 microliter) is required, and the meter requires frequent calibration.[44]

Most SMBG meters today utilize an electrochemical or enzyme electrode process, which determines glucose levels by measuring an electric charge produced by the glucose-reagent reaction. These second-generation glucometers can further be subdivided according to the electrochemical principle used: amperometry or colorimetry.

Amperometry biosensor technology requires a large sample size (4–10 μL). Amperometric technology measures only a small percentage of the glucose and uses a multiplier to convert this to a numerical value. Therefore, blood glucose readings may be affected by environmental temperature, hematocrit, medications, and other factors. Also, small samples may result in inaccurate readings because of a weak signal being generated. The Sidekick Testing System employs the amperometry method.[43,44]

The *colorimetry* method involves converting the glucose sample into an electrochemical charge, which is then captured for measurement. An advantage of this system is that a small amount of blood (e.g., 0.3 mL) is enough to determine the blood glucose level. The colorimetry method is not influenced by changes in temperature and hematocrit levels. These monitors can use blood samples extracted from the arm and thigh too. At these alternative sites, there are fewer capillaries and nerve endings, allowing for a less painful needle stick. Some examples of second-generation glucometers that use the colorimetry method include ReliOn Ultima, OneTouch Ultra, and Freestyle. A chart which lists the current glucose meters and their features can be found at http://main.diabetes.org/dforg/pdfs/2015/2015-cg-glucose-meters.pdf.

Although new SMBG meters report results as plasma values, older meters may report results as whole blood values, which are approximately 10–15% lower than plasma values. The ADA recommends whole blood fasting readings of 80–130 mg/dL (4.4–6.7 mmol/L) and bedtime readings between 100–140 mg/dL (5.6–7.8 mmol/L). Plasma fasting readings should be between 90–130 mg/dL (5–7.2 mmol/L) and bedtime readings between 110–140 mg/dL (6.1–7.8 mmol/L).[28]

Special features of glucose meters. Blood glucose testing can be challenging for adults with poor vision or limited dexterity as well as children with small hands. Patients should try out several meters by checking their ease of use with the lancing device and lancets, test strips, and packaging, and meter features before committing to one. Meters with a backlight or test strip port light should be recommended for individuals with visual impairment. Many meters also offer an audio function that is available in several different languages. Persons with limited dexterity, such as patients with arthritis, neuropathy, Parkinson disease, etc., may consider either the Breeze 2, which has a disk that holds 10 strips, or the Accu Check Compact Plus meter, which holds a drum of test strips. The test strip for these meters is easily dispensed with a push of a button. Individuals with visual impairment may benefit from larger display screen, screen back light, or test strip port light. Examples of meters that offer a screen back light include the Freestyle Lite, One-Touch Ultra 2, and ReliOn Ultima. The Contour Next USB and Freestyle Lite meters also have test strip port lights.[45]

Some children are more comfortable monitoring their blood glucose than others. Some children may like glucometers that come in bright or "cool" colors. Many "auto-code" or "no-code" meters, which do not require manually programming the meter to recognize a specific group of test strips, are ideal choices for children who are learning how to monitor their blood glucose levels. Parents should select a meter that requires a very small blood sample size.

Most meters hold from 100 to 450 test results, though a few save well over 1000. This makes it easier to track blood glucose control over time. Many meters on the market have computer download capability through a USB connection. These meters come with their own software that can be downloaded directly to a MAC or PC desktop computers. Meter results can also be exported to an Excel file.[45]

An additional method to assess glucose control is called *continuous glucose monitoring,* which provides readings every few minutes throughout the day. This method allows patients and providers an opportunity to observe trends in glucose levels throughout the day and make the appropriate adjustments to medication, meal, or exercise regimens. A small sterile disposable glucose-sensing device called a *sensor* is inserted into the subcutaneous tissue. This sensor measures the change in glucose levels in interstitial fluid and sends the information to a monitor that can store three to seven days of data. The monitor must be calibrated daily by entering at least three blood glucose readings obtained at different times using a standard blood glucose meter. The monitors have an alert system to warn patients if their blood glucose level is dangerously low or high. The current monitoring systems include the G4 Platinum (manufactured by Dexcom), Guardian Real-Time MiniMed 530G with Enlite, and MiniMed Paradigm Real-Time Revel (manufactured by Medtronic Diabetes).[46]

Generally, blood glucose concentrations determined by these methods are clinically useful estimates of corresponding plasma glucose concentrations measured by the laboratory. Therefore, home blood glucose monitoring is preferred to urine testing. Home blood testing clarifies the relationship between symptomatology and blood glucose concentrations. The best meter for a patient is an individual decision. Patients should be encouraged to try different brands of meters to find the device with which they are most comfortable.[43]

Quality control, which consists of control solution testing, calibration, and system maintenance, is a necessary component of accurate glucose testing. Most manufacturers supply control solutions with SMBG meters that can be used to assess the accuracy of the test strip. This method of verifying accuracy operates the same way that the patient analyzes a drop of blood. A few meters require manual calibration prior to use, but most have an automatic calibration mode for ease of use. In photometric meters, the blood sample intended for the strip may come in contact with the meter and soil the optic window resulting in inaccurate results. Pharmacists should guide patients through the instructions for cleaning the meter that are usually provided by the manufacturer.

Factors affecting glucose readings. Environmental factors such as temperature, humidity, altitude, and light may influence the accuracy of glucose readings. Exposing glucometers to extremes of temperature can alter battery life and performance. Therefore, glucometers should be stored at room temperature to ensure accuracy (most will function at temperatures between 50 °F and 104 °F). The ReliOn Precision Xtra will function at higher temperatures (122 °F maximum), while the Sidekick glucose meter is ideal for lower temperatures (34 °F minimum).[45,48] Temperature changes and humidity may decrease the shelf life of test strips resulting in inaccurate test results. Test strips should not be stored in areas of high humidity, such as a bathroom, or in areas with notable temperature changes, such as the car. Individuals should check the expiration date of the test strips. Because test strips are costly, patients are often tempted to use expired strips that result in inaccurate readings.[47] Most test strips expire within 90–180 days after being opened.

At higher altitudes, changes in oxygen content and temperature alter glucose testing results. Results of studies evaluating the accuracy of glucometers at altitudes >10,000 feet have revealed major alterations in blood glucose levels. These changes are attributed to variations in metabolic rate, hydration, diet, physical exercise, hematocrit, and temperature associated with higher altitudes. Patients should be educated on how to use glucometers at high altitudes. Changes in light exposure can also alter results with photometric glucometers.[43] Additional variables such as hypotension, hypoxia, high triglyceride concentrations, and various drugs can alter readings; each patient should be evaluated for the presence of such variables and medication-related effects. User error is the most common reason for inaccurate results. Some of the most common errors include not putting enough blood on the reagent portion of the strip. Patients should be asked periodically to demonstrate how they operate the meter.[44,47]

Accuracy of glucose readings. The U.S. Food and Drug Administration (FDA) requires 95% of all meter test results to be within 20% of the actual blood glucose level for results at or above 75 mg/dL (4.2 mmol/L). So an actual blood glucose that is 100 mg/dL (5.6 mmol/L) could show on a meter as being between 80 and 120 mg/dL (4.4–6.7 mmol/L) and still be considered accurate. The FDA is currently reviewing more stringent standards that will require 99% of meter test results to be within 15% of the actual blood glucose level for results at or above 75 mg/dL (4.2 mmol/L). For example, an actual blood glucose result of 100 mg/dL (5.6 mmol/L) could potentially show on a meter as any value between 85 and 115 mg/dL (4.7–6.4 mmol/L) and meet the standard.

The guidelines for results in the hypoglycemic range, defined as a blood glucose level below 75 mg/dL (4.2 mmol/L), stipulate that 99% of test results must be within ± 15 mg/dL of the actual blood glucose level. Therefore, if an actual blood glucose level is 60 mg/dL, the guidance says the reading would need to be between 45 and 75 mg/dL (2.5–4.2 mmol/L) to meet accuracy standards.

The FDA guidance also recommends that meter boxes and test strip vials include easy-to-understand accuracy data—both on the outside of the package and on the insert inside The FDA does not regularly monitor blood glucose meters or strips once they enter the commercial market. This means some companies may not maintain the same level of quality and accuracy as when the products were initially approved.[49,50]

Frequency of glucose monitoring. Glucose monitoring requirements may vary based on the pharmacologic therapy administered. Patients who are well-controlled on oral medications should monitor blood glucose levels at least daily and more frequently if there are any changes in drugs or drug doses. Patients who are poorly controlled on oral medications should monitor blood glucose levels two to four times daily. Patients taking insulin injections twice a day should check blood glucose levels at least twice a day. Patients on intensive insulin therapy should monitor blood glucose levels three to four times a day. Patients on insulin pumps need monitoring four to six times a day to determine the effectiveness of the basal and bolus doses. In general, premeal glucose measurements are needed to monitor the effectiveness of the basal insulin dose (e.g., glargine or detemir) dose. Two-hour PPG readings are needed to monitor rapid-acting insulins (e.g., lispro, glulisine, aspart and Afrezza inhaled). Oral blood glucose lowering agents, such as metformin, thiazolidinediones, sitagliptin, and glipizide, are evaluated using two-hour postprandial readings. Pregnancy requires frequent monitoring of blood glucose levels four to six times a day to ensure tight control. Premeal testing is required during acute illness to determine the need for supplemental insulin.[51-58]

Diagnosis of Hyperglycemia

The diagnosis of patients with *hyperglycemia* commonly falls into one of three categories: (1) DM or prediabetes, (2) DKA, and (3) hyperosmolar hyperglycemia state (HHS).[28]

Diabetes Mellitus or Prediabetes

Goals of therapy. Once DM is diagnosed, the clinician needs to establish a therapeutic goal with respect to glucose control. The ideal goal would be to maintain concentrations at normal physiological levels. For type 1 patients, this effort requires tight control. Tight control could include preprandial plasma glucose concentrations of 80–130 mg/dL (4.4–7.2 mmol/L) and postprandial concentrations of <180 mg/dL (<10 mmol/L). Tight control requires intensive therapy and monitoring (i.e., three or more insulin injections per day and self-monitoring of glucose concentrations).[28]

Data from the Diabetes Control and Complications Trial suggest that this approach leads to significant health benefits.[59] Overall, the study showed that in patients with type 1 DM, intensive therapy delays the onset and slows progression of microvascular complications (e.g., diabetic retinopathy, nephropathy, and neuropathy), and macrovascular diseases (e.g., large vessel, peripheral vascular diseases, stroke, and ischemic heart disease).

The United Kingdom Prospective Diabetes Study (UKPDS 33) compared the effects of intensive drug therapy with a sulfonylurea (e.g., chlorpropamide and glipizide) insulin and conventional therapy (diet) in 3867 patients with newly diagnosed type 2 DM from 1977 until 1991.[60] The results of the UKPDS show that intensive drug therapy decreased the risk of microvascular complications when compared with conventional therapy. The investigators reported that patients in the intensive group with a 1% median reduction in A1c had a 25% reduction in risk of microvascular complications ($p = 0.0099$).

Basal secretion of insulin from the pancreas occurs at a rate of 0.5–1 unit/hr. Bolus secretion of insulin occurs when blood sugar levels are above 100 mg/dL (5.6 mmol/L) after ingestion of food. Therefore, the normal physiologic release pattern of insulin is one of valleys or basal secretion and peaks or bolus secretions. The normal pancreas secretes 25–50 units of insulin daily.[61,62]

Because the most common and effective means to manage insulin deficiency is with insulin, the importance of the relationship between insulin injection and interpretation of glucose concentrations is briefly discussed. In general, 1 unit of any type of insulin produces the same metabolic response. An increase of 1–2 units in the insulin dose usually leads to a decrease of 30–50 mg/dL in the glucose concentration. Although the amount of total activity is equal for any given number of units of any insulin, the activity is not elicited evenly over time. A clinician must know the activity-over-time profiles of insulin types at various injection sites to anticipate correctly a dose's effect on glucose concentrations at a particular time (**Table 10-4** and **Figure 10-2**).[61]

TABLE 10-4. Comparisons of Human Insulins and Insulin Analogues

INSULIN	ONSET (min)	PEAK ACTION (hr)	DURATION (hr)
Rapid-acting			
Lispro/aspart/glulisine	5–15	0.5–1.5	4–6
Regular insulin inhalation powder (Afrezza)	12–15	60	2.5–3
Short-acting			
Human regular	30–60	2–4	6–10
Intermediate-acting			
Human NPH	60–120	4–8	10–18
Combinations			
70/30; 50/50; 75/25	30–60	2–8	14–18
Long-acting			
Detemir U 100	60–180	6–8	6–23
Glargine U 100	60–180	Peakless	24
Degludec U 200	30–90	Peakless	40
Glargine U 300	60–180	Peakless	36–42

hr = hours; min = minutes; NPH = neutral protamine Hagedorn; U = units.

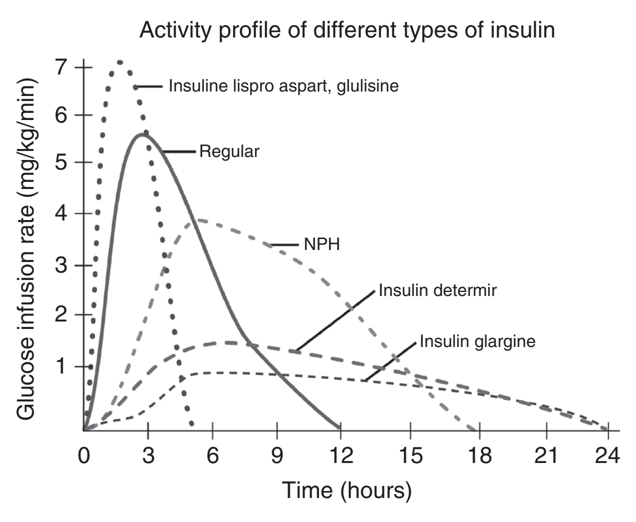
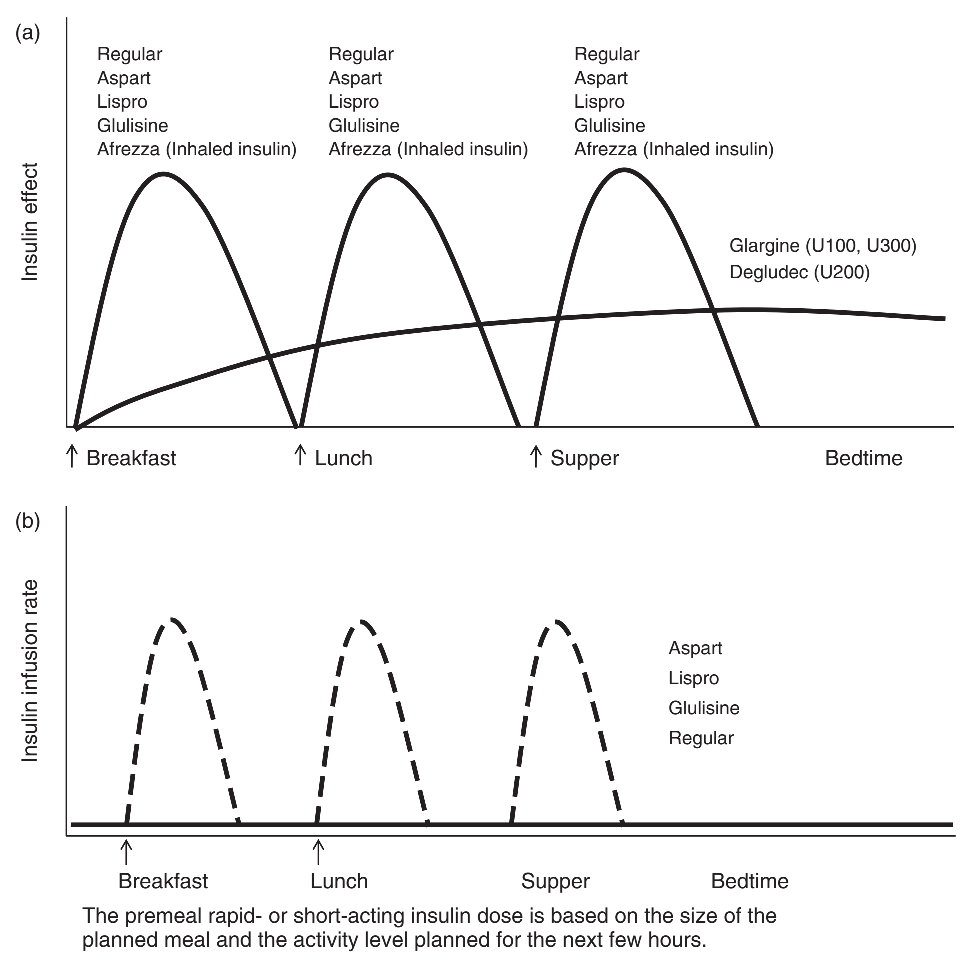


FIGURE 10-2. Pharmacokinetic activity profiles of different types of prescribed insulins and insulin analogues. *Source:* These illustrations are made available by written permission of The Regents of the University of California. All rights reserved. Adapted from www.dtc.ucsf.edu. Made available by written permission from the University of California. All rights reserved.

The starting dose of insulin is based on clinical assessment of insulin deficiency and insulin resistance, as well as the patient's lifestyle (i.e., eating patterns, exercise, and waking/sleep patterns). In general, insulin requirements for patients with type 1 DM with a BMI <25 kg/m^2 are 0.5–1 unit/kg/day. Insulin requirements may vary during illness or stress. Insulin requirements may decrease during the "honeymoon phase," a remission period that occurs early in the course of the disease.[28] Insulin requirements for patients with type 2 DM vary based on the degree of insulin resistance and insulin deficiency. In general, a single or bedtime dose of insulin can be given to patients with type 2 DM who do not achieve glycemic control with noninsulin agents. The noninsulin agents should be continued at the same dose in most cases. Patients with a BMI <25 kg/m^2 can be started on 5–10 units of intermediate or long-acting insulin at bedtime. Patients with a BMI >25 kg/m^2 should be started on (1) 10–15 units of intermediate (NPH), long-acting (glargine U100 or detemir), or ultralong-acting (glargine U300 or degludec U200) insulin at bedtime, or (2) combination insulin (70/30) before dinner.[64]

Figure 10-3a illustrates the use of (1) a short-acting insulin (regular) or a rapid-acting insulin (lispro, glulisine, aspart insulin, or regular insulin inhalation powder [Afrezza]) given before breakfast, lunch, and dinner, or (2) a long-acting basal insulin (detemir or glargine U100) or an ultralong-acting basal insulin (glargine U300 or degludec U200) given at breakfast. This regimen, often referred to as "the poor man's pump," is ideal for patients with unusual schedules. **Figure 10-3b** illustrates insulin therapy administered by an insulin pump. The pump is programmed to administer insulin (rapid-acting or short-acting) throughout the day, and the patient activates the pump to give a bolus of insulin prior to meals. Intensive regimens require more frequent monitoring and are accompanied by an increased risk of hypoglycemia.

In a single-daily regimen, an injection of ultralong-acting insulin or a combination injection of intermediate and long-acting insulin is administered at bedtime. This regimen is ideal for a patient with type 2 DM who has failed to obtain glycemic control on oral agents. The oral agents can be continued, and the combination of intermediate and long-acting insulin



The premeal rapid- or short-acting insulin dose is based on the size of the planned meal and the activity level planned for the next few hours.

FIGURE 10-3A, 3-B. Current intensive insulin regimens for diabetes. *Source:* These illustrations are made available by written permission of The Regents of the University of California. All rights reserved. Adapted from www.dtc.ucsf.edu. Made available by written permission of the University of California. All rights reserved.

can be administered at bedtime. In a twice-daily regimen, an injection of intermediate-acting insulin is administered before breakfast and at bedtime. The administration of intermediate-acting insulin before bedtime decreases the risk of hypoglycemia in the early morning.

An average regimen combines a mixed injection of intermediate-acting and regular insulin given before breakfast, an injection of regular insulin at dinner, and another injection of intermediate-acting insulin given before bedtime, which provides safer, more effective overnight glucose control. Without regular insulin administered before dinner, however, glucose concentrations may become unacceptably high after dinner.

Regular insulin should be given 30–45 minutes before meals unless the preprandial glucose concentration <70 mg/dL (<3.9 mmol/L). If the concentration is <70 mg/dL, the patient should consume 15 g of carbohydrates (e.g., 4 ounces of orange juice, regular soda, or skim milk) immediately, and check glucose levels 15 minutes later. In all regimens, regular insulin doses are adjusted on the basis of self-monitored preprandial glucose concentrations. Regular insulin inhalation powder (Afrezza) and lispro, glulisine, or aspart insulin should be given 5–15 minutes before a meal. Insulin should be used along with diet, exercise, and stress management for best effects.[62-64]

Oral agents in the management of type 2 DM. Currently, there are several classes of oral agents available for the management of diabetes. All lower blood glucose concentrations in responsive patients (**Appendix A**).

Diabetic Ketoacidosis

Insulin deficiency after ingestion of a meal can result in impaired glucose utilization by peripheral tissues and the liver. Prolonged insulin deficiency results in protein breakdown and increased glucose production (gluconeogenesis) by the liver and increased release of counter regulatory hormones such as glucagon, catecholamines (e.g., epinephrine and norepinephrine), cortisol, and growth hormones. In the face of lipolysis, free fatty acids are converted by the liver to ketone bodies (β-hydroxybutyric acid and acetoacetic acid), which results in metabolic acidosis. Diabetic ketoacidosis (DKA), which occurs most commonly in patients with type 1 DM, is initiated by insulin deficiency (**Minicase 1**). The most common causes of DKA include the following[70]:

- Infections, illness, and emotional stress
- Nonadherence or inadequate insulin dosage
- Undiagnosed type 1 DM
- Unknown or no precipitating event

Clinically, patients with DKA typically present with dehydration, lethargy, acetone-smelling breath, abdominal pain, tachycardia, orthostatic hypotension, tachypnea, and, occasionally, mild hypothermia, and lethargy or coma. Because of the patient's tendency toward low body temperatures, fever strongly suggests infection as a precipitant of DKA. Chemically, DKA is characterized by a high-glucose concentration. This concentration is typically >250 mg/dL or 13.9 mmol/L. As insulin is given, the glucose concentrations decline at a rate of about 75–100 mg/dL/hr.[71]

DKA also characterized by low venous bicarbonate (0–15 mEq/L) and a decreased arterial pH (<7.0–7.2). Hyperglycemia can lead to osmotic diuresis resulting in hypotonic fluid losses, dehydration, and electrolyte loss. Sodium and potassium concentrations may be low, normal, or high. Sodium concentrations are reflective of the amount of total body water and sodium lost and replaced. In the presence of hyperglycemia, sodium concentrations may be decreased because of the movement of water from the intracellular space to the extracellular space. The potassium level reflects a balance between the amount of potassium lost in the urine and insulin deficiency, which causes higher concentrations of serum potassium as potassium shifts from intracellular spaces to extracellular fluid. Hypertonicity and acidosis can cause potassium to move from the intracellular space to the extracellular space, resulting in elevated potassium levels. However, total potassium depletion always occurs regardless of the initial potassium level. Patients with low or normal potassium levels should be monitored closely because treatment can result in a severe total body potassium deficit that may place the patient at risk for cardiac dysrhythmia.

The phosphate level is usually normal or slightly elevated.[70, 72] Creatinine and blood urea nitrogen (BUN) are usually elevated due to dehydration. These levels usually return to normal after rehydration unless there was pre-existing renal insufficiency. Hemoglobin, hematocrit, and total protein levels are mildly elevated due to decreased plasma volume and dehydration. Amylase levels may be increased due to increased secretion by the salivary glands. Liver function tests are usually elevated but return to normal in three to four weeks.

Initially, potassium concentrations may be elevated due to metabolic acidosis. Correction of acidosis elicits the opposite effect. Potassium shifts intracellularly with insulin therapy, leading to a decrease in serum potassium concentrations. Serum osmolality is typically elevated at 300–320 mOsm/kg (normally, 280–295 mOsm/kg). Serum osmolarity (milliosmoles per liter), which is practically equivalent to osmolality (milliosmoles per kilogram), can be estimated using the following formula:

$$\text{serum osmolarity (mOsm/L)} = (2 \times \text{sodium}) + \text{glucose}/18 + \text{BUN}/2.8$$

where glucose and BUN units are expressed as milligrams per deciliter.

Blood and urine ketones. Ketones are present in the blood and urine of patients in DKA, as the name of this disorder implies. Formation of ketone bodies results from fat metabolism. Three principal ketone bodies include acetoacetate, acetone, and β-hydroxybutyrate, which is the predominate ketone in the blood of patients with DKA.[72,73] DKA can be prevented if patients are educated about detection of hyperglycemia and ketonuria. It is recommended that all patients with DM test their urine for ketones during acute illness or stress when blood glucose levels are consistently >250 mg/dL (14 mmol/L), during pregnancy, or when any symptoms of ketoacidosis—such as nausea, vomiting, or abdominal pain—are present.[73,74]

MINICASE 1

Euglycemic Diabetic Ketoacidosis

Vera K. is a 45-year-old female with a two-year history of type 2 DM. She presents to the ED with a pH of 7.25, HCO_3 of 9, and blood glucose of 180 mg/dL. Her husband brought her into the ED after finding her "out of it" when attempting to wake her. She was vomiting and unable to eat over the last 24 hours, and she began experiencing labored breathing, fever, chills, and unusual fatigue.

Approximately one year ago, her provider started her on Janumet (sitagliptin–metformin, 50 mg/1000 mg) at breakfast and dinner. At her most recent clinic appointment one month ago, her laboratory results indicated that her HbA1c has dropped from 9.2 to 7.8 since starting sitagliptin–metformin. Her doctor decided to add dapagliflozin 10 mg to her regimen to obtain an HbA1c below 7%. Vera K. also takes atorvastatin 40 mg at bedtime for elevated cholesterol as well as lisinopril 10 mg daily and hydrochlorothiazide 25 mg daily for hypertension. She has tolerated all of her medications and adheres to the indicated daily schedule. Physical examination reveals a lethargic female with vital signs including BP 116/68 mm Hg (which dropped to 95/50 when standing); HR 105 beats/min; RR 30 breaths/min (deep and regular); and oral temperature 101.4 °F (38.6 °C). Her skin turgor is poor, her mucous membranes are dry, and she is disoriented and confused. Her laboratory results are as follows:

- Sodium, 142 mEq/L (136–142 mEq/L)
- Potassium, 5 mEq/L (3.8–5 mEq/L)
- Chloride, 99 mEq/L (95–103 mEq/L)
- BUN, 28 mg/dL (8–23 mg/dL)
- SCr, 1.8 mg/dL (0.6–1.2 mg/dL)
- Phosphorus, 2.7 mg/dL (2.3–4.7 mg/dL)
- Amylase, 200 IU/L (30–220 IU/L)
- pH, 7.25 (7.38–7.44)
- Bicarbonate, 9 mEq/L (21–280 mEq/L)
- Hct, 52% (36–45%)
- WBC count, 16 × 10^3 cells/mm^3 (4.8–10.8 × 10^3 cells/mm^3)
- Calcium, 9 mg/dL (9.2–11 mg/dL)
- Glucose, 180 mg/dL (70–110 mg/dL)
- Ketones, 3+ @1:8 serum dilution (normal = 0)
- Osmolality, 304 mOsm/kg (280–295 mOsm/kg)
- Triglycerides, 174 mg/dL (10–190 mg/dL)
- Lipase, 1.4 units/mL (<1.5 units/mL)
- Magnesium, 2 mEq/L (1.3–2.1 mEq/L)

A urine screen with Multistix indicates a large (160 mg/dL) amount of ketones (the highest designation on the strip).

QUESTION: Based on clinical and laboratory findings, what is the most likely diagnosis for this patient? What precipitated this metabolic disorder? Can interpretation of any results be influenced by her acidosis or hyperglycemia? Are there potential medication interferences with any laboratory tests?

DISCUSSION: She is a type 2 DM patient and presents with DKA, which is not typically observed in patients with type 2 DM. Since 2014, the FDA has received over 20 reports of DKA in patients treated with SGLT2 inhibitors. The FDA reports that DKA case presentations are atypical in that glucose levels are mildly elevated at <200 mg/dL, while patients with type 1 DM typically have glucose levels >300 mg/dL. This patient does not have any potential DKA-triggering factors that include acute illness changes, such as infection (e.g., urinary tract infection, gastroenteritis, influenza, or trauma), urosepsis, trauma, reduced caloric or fluid intake, and reduced insulin dose—all of which do not apply to her case. The cause of preserved euglycemia could be greater urinary loss of glucose triggered by counter-regulatory hormones, hepatic glucose production observed during a fasting state, or the SGLT2 inhibitor. A key physiologic determinant is the quantity of food she ingested prior to development of diabetic ketoacidosis. That is, when patients are well fed, their liver contains large amounts of glycogen, which primes the liver to produce glucose and to suppress ketogenesis. However, when patients have been vomiting and unable to eat, the liver is depleted of glycogen and primed to produce ketones. Thus, patients like this one with euglycemic ketoacidosis are usually in the fasting state before they become ill.

Clinically, this patient presents with typical signs of ketoacidosis, which include difficulty breathing, nausea, vomiting, abdominal pain, confusion, and unusual fatigue and sleepiness. Her decreased skin turgor, dry mucous membranes, tachycardia (HR 105 beats/min), and orthostatic hypotension are consistent with dehydration, a common condition in patients with DKA. Her breathing is rapid and deep. Although she is not comatose, she is lethargic, confused, and disoriented. Chemically, she probably has a total body deficit of sodium and potassium despite serum concentration results within normal limits.

Orthostatic hypotension is consistent with decreased intravascular volume, causing hemoconcentration of these electrolytes. Therefore, these values do not reflect total body stores, and the clinician can expect them to decline rapidly if unsupplemented fluids are infused. Although her phosphorus concentration is in the normal range (lower end), it likely will decrease after rehydration and insulin. Serial testing should be done every three to four hours during the first 24 hours.

Serial glucose, ketones, and acid–base measurements, typical of DKA, should show gradual improvement with proper therapy. Potassium balance is altered in patients with DKA because of combined urinary and GI losses. Although total potassium is depleted, the serum potassium concentration may be high, normal, or low, depending on the degree of acidosis. Her metabolic acidosis has resulted in an extracellular shifting of potassium, causing an elevated serum potassium concentration. Potassium supplementation may be withheld for the first hour or until serum levels begin to drop. Low serum potassium in the face of pronounced acidosis suggests severe potassium depletion requiring early, aggressive therapy to prevent life-threatening hypokalemia during treatment.

Decreased intravascular volume has led to a hemoconcentrated hematocrit and BUN, which is also elevated by decreased renal perfusion (prerenal azotemia), although intrinsic renal causes should be considered if SCr is also elevated. Fortunately, as is probably the case with this patient, high SCr may be an artifact caused by the influence of ketone bodies on the assay. If so, SCr concentrations should decline with ketone concentrations.

She also exhibits the typical leukocytosis that often accompanies DKA in the absence of infection. Her estimated plasma osmolarity based on the osmolarity estimation formula would be (2 × 142) + (180/18) + (28/2.8) = 304 mOsm/L, is approximately equal to the actual measured laboratory result.

The serum ketone results still would have to be interpreted as real and significant, given all of the other signs and symptoms. A urine screen also indicated the presence of ketones.

All of the commercially available urine testing methods are based on the reaction of acetoacetic acid with sodium nitroprusside (nitroferricyanide) in a strongly basic medium. The colors range from beige or buff-pink for a “negative” reading to pink and pink-purple for a “positive” reading (Acetest, Ketostix, Laboratorystix, and Multistix). These nitroprusside-based (nitroferricyanide) assays do not detect β-hydroxybutyric acid and are 15–20 times more sensitive to acetoacetate than to acetone. In a few situations (e.g., severe hypovolemia, hypotension, low partial pressure of oxygen [pO_2], and alcoholism) where β-hydroxybutyrate predominates, assessment of ketones may be falsely low. As DKA resolves, β-hydroxybutyric acid is converted to acetoacetate, the assay-reactive ketone body. Therefore, a stronger reaction may be encountered in laboratory results. However, this reaction does not necessarily mean a worsening of the ketoacidotic state.[73]

Clinicians must keep in mind that ketonuria may also result from starvation, high-fat diets, fever, and anesthesia, but these conditions are not associated with hyperglycemia. Levodopa, mesna, acetylcysteine (irrigation), methyldopa, phenazopyridine, pyrazinamide, valproic acid, captopril, and high-dose aspirin may cause false-positive results with urine ketone tests.[75-78,81,82] The influence of these drugs on serum ketone tests has not been studied extensively. If the ketone concentration is increased, a typical series of dipstick results includes the following:

1. Negative
2. Trace or 5 mg/dL
3. Small or 15 mg/dL
4. Moderate or 40 mg/dL
5. Large or 80 mg/dL
6. Very large or 160 mg/dL

False-negative readings have been reported when test strips have been exposed to air for an extended period of time or when urine specimens have been highly acidic, such as after large intakes of ascorbic acid.[34] Urine ketone tests should not be used for diagnosing or monitoring treatment of DKA. Acetoacetic and β-hydroxybutyric acids concentrations in urine greatly exceed blood concentrations. Therefore, the presence of ketone bodies in urine cannot be used to diagnose DKA. Conversely, during recovery from ketoacidosis, ketone bodies may be detected in urine long after blood concentrations have fallen.[34,81] In addition, urine testing only provides an estimate of blood ketone levels two to four hours before testing and is dependent on the person being able to pass urine.

The ADA recommends testing blood β-ketone because ketones are detectable in the blood far earlier than in urine, so blood β-ketone testing can provide a patient with an early warning of impending DKA.[74] A β-hydroxybutyric acid level <0.6 mmol/L is considered normal. Patients with levels between 0.6 and 1 mmol/L should take additional insulin and increase their fluid intake to flush out the ketones. Patients should contact their physician if levels are between 1–3 mmol/L. Patients should be advised to report to the emergency department immediately if their levels are >3 mmol/L.[78,79]

Hyperosmolar Hyperglycemia State

Hyperosmolar hyperglycemia state (HHS) is a condition that occurs most frequently in elderly patients with type 2 DM, and it is usually precipitated by stress or illness when such patients do not drink enough to keep up with osmotic diuresis. Patients usually present with severe hyperglycemia (glucose concentrations >600 mg/dL or >33.3 mmol/L); decreased mentation (e.g., lethargy, confusion, dehydration); neurologic manifestations (e.g., seizures and hemisensory deficits); and an absence of ketosis. Insulin deficiency is not as severe in HHS as in DKA. Therefore, lipolysis—which is necessary for the formation of ketone bodies—does not occur (**Minicase 2**). The absence of ketosis results in significantly milder GI symptoms than patients with DKA. Therefore, patients often fail to seek medical attention. Patients with HHS are usually more dehydrated on presentation than patients with DKA due to impairment in the thirst mechanism, which results in prolonged diuresis and dehydration.[72]

In some cases, patients are taking medications that cause glucose intolerance (e.g., diuretics, steroids, and phenytoin). Stroke and infection are disease-related predisposing factors. Initially, electrolytes are within normal ranges, but BUN routinely is elevated. Serum osmolalities characteristically are higher than those in DKA—in the range of 320–400 mOsm/kg. Serum electrolytes (e.g., magnesium, phosphorus, and calcium) are typically abnormal and should be monitored until they return to normal range.[71-73]

Hypoglycemia

Hypoglycemia is defined as a blood glucose level of 70 mg/dL (3.9 mmol/L) or lower. The classification of hypoglycemia is based on the individual’s ability to self-treat. Mild hypoglycemia is characterized by symptoms such as sweating, trembling, shaking, rapid heartbeat, heavy breathing, and difficulty concentrating. The symptoms associated with mild hypoglycemia vary in severity and do not imply that the symptoms experienced by the individual are minor or easily tolerated. Although patients may experience profuse sweating, dizziness, and lack of coordination, they still may be able to self-treat. These symptoms resolve after consuming carbohydrates (e.g., fruit juice, milk, or hard candy).[82,83]

Severe hypoglycemia is characterized by an inability to self-treat due to mental confusion or unconsciousness. Emergency medical treatment is required to raise the blood glucose level out of a dangerously low range.[83] Increased release of counter regulatory hormones is responsible for most hypoglycemic symptoms. Most of the early signs of hypoglycemia (e.g., trembling, shaking, rapid heartbeat, fast pulse, heavy breathing, and changes in body temperature) are mediated by the adrenergic system. Sweating, another cardinal sign of hypoglycemia, is mediated by the cholinergic system.[84]

Glucagon and epinephrine are the primary counter regulatory hormones responsible for increasing blood glucose concentrations in the presence of hypoglycemia. Glucagon enhances glycogenolysis and epinephrine increases

MINICASE 2

Hyperosmolar Hyperglycemia State Secondary to Uncontrolled Type 2 DM

Jimmy C. is a 63-year-old African-American male with a 19-year history of type 2 DM, hypertension, and dyslipidemia. He lives alone. His medication lists includes metformin 1000 mg BID, simvastatin 20 mg daily at bedtime, lisinopril 20 mg daily, hydrochlorothiazide 25 mg daily, and ASA 325 mg daily. He monitors his blood glucose once a day, and his results have ranged from 215–400 mg/dL. His fasting blood glucose has averaged 200 mg/dL over the last week. He reports frequent urination throughout the day and night, which has increased over the last six days. He denies any nausea or vomiting but states he hasn't had much of an appetite lately. His daughter accompanies him to the doctor's office because she thinks he has been acting weird lately.

The physical examination reveals a very disoriented and confused man with vital signs including BP 120/60 mm Hg (which dropped to 100/60 when standing); HR 100 beats/min; RR 20 breaths/min (deep and regular); and oral temperature 101.4 °F (38.6 °C). His skin turgor is poor, and his mucous membranes are dry. His laboratory results are as follows:

- Sodium, 139 mEq/L (136–142 mEq/L)
- Potassium, 4.6 mEq/L (3.8–5 mEq/L)
- Chloride, 102 mEq/L (95–103 mEq/L)
- BUN, 50 mg/dL (8–23 mg/dL)
- SCr, 1.2 mg/dL (0.6–1.2 mg/dL)
- Phosphorus, 2.7 mg/dL (2.3–4.7 mg/dL)
- pH, 7.38 (7.36–7.44)
- Bicarbonate, 26 mEq/L (21–28 mEq/L)
- Hct, 39% (36–45%)
- WBC count, 9.4 × 10^3 cells/mm^3 (4.8–10.8 × 10^3 cells/mm^3)
- Calcium, 9 mg/dL (9.2–11 mg/dL)
- Glucose, 715 mg/dL (70–110 mg/dL)
- Ketones, 0 (normal = 0)
- Osmolality, 335 mOsm/kg (280–295 mOsm/kg)
- Triglycerides, 174 mg/dL (10–190 mg/dL)
- Lipase, 1.4 units/mL (<1.5 units/mL)
- Magnesium, 2 mEq/L (1.3–2.1 mEq/L)
- Hemoglobin A1c, 9.5 (4–5.6%)

QUESTION: Based on the subjective and objective data provided, what is the most likely diagnosis for this patient? What signs and symptoms support the diagnosis? What could have precipitated this disorder?

DISCUSSION: He is over 60 years of age. Hyperosmolar hyperglycemia state occurs most frequently in patients over 60 years of age. He complains of symptoms for more than five days. He has decreased skin turgor, dry mucous membranes, tachycardia (HR 100 beats/min), and orthostatic hypotension (a fall of systolic BP 20 mm Hg after one minute of standing), which are consistent with dehydration. He is lethargic, confused, and disoriented. HHS patients are generally more dehydrated; therefore, mentation changes are more commonly seen in HHS than in DKA. The elderly often have an impaired thirst mechanism that increases the risk of HHS. His plasma glucose level is >600 mg/dL; bicarbonate concentration is normal; and pH is normal. Negative ketone bodies <2+ in 1:1 dilution confirms the diagnosis of HHS (and not DKA where ketones are present in the blood and urine of patients). Insulin deficiency is less profound in HHS; therefore, lipolysis resulting in the production of ketone bodies does not occur. His plasma osmolarity can be estimated using a formula:

$$pOsm= (2 \times \text{serum sodium}) + \text{glucose}/18 + \text{BUN}/2.8$$

The estimated osmolality is (2 × 139) + (715/18) + 50/2.8 = 335 mOsm/kg, which is the same as the actual laboratory value. Massive fluid loss due to prolonged osmotic diuresis secondary to hyperglycemia may have precipitated the onset of HHS.

He should be rehydrated with oral fluids because he has no complaints of GI discomfort. Insulin should be administered. Although his sodium and potassium are within normal limits, the presence of orthostatic hypotension is consistent with decreased intravascular volume, causing hemoconcentration of sodium and potassium. Potassium shifts out of cells when the pH of the blood is acidic due to an increased influx of hydrogen ions. The total potassium concentration appears normal because potassium has shifted from the intracellular compartment to the circulation. These levels may decline when the patient is rehydrated with fluids. Potassium replacement will probably be required. Phosphorus is also within normal limits but may decrease after rehydration and insulin. Decreased intravascular volume has led to hemoconcentration of hematocrit and BUN, which is also elevated because of decreased renal perfusion (prerenal azotemia), although intrinsic renal causes should be considered if SCr is also elevated.

Metformin alone or in combination with any oral antidiabetic agent will not lower his A1C to a goal of <7%. One injection of long-acting insulin administered at breakfast or bedtime is an ideal regimen for a patient with type 2 DM who has failed to obtain glycemic control on oral agent(s). Metformin can be continued if the serum creatinine returns to normal and long-acting insulin can be administered at bedtime.

gluconeogenesis and inhibits glucose utilization by tissues. Defects in hormonal counter regulation can diminish autonomic symptoms resulting in hypoglycemia unawareness. Glucagon secretion may become impaired after the first few years of type 1 DM, resulting in epinephrine as the primary mechanism for raising low blood glucose levels. Frequent episodes of hypoglycemia can cause temporary deficits in epinephrine response, resulting in an absence of autonomic symptoms for several days. Epinephrine response returns to normal when patients avoid low blood glucose levels over a period of three to six weeks. Patients with diabetes must be taught the importance of maintaining a balanced diet and monitoring their blood glucose levels regularly to decrease the risk of developing hypoglycemia.[83,84]

Neuroglycopenia occurs during hypoglycemic episodes due to decreased glucose supply to the central nervous system. The earliest signs of neuroglycopenia include slow thinking and difficulty concentrating and reading. Patients report that it takes more effort to perform routine tasks (e.g., brushing teeth, combing hair, or taking a bath). As the blood glucose levels decrease further, mental confusion, disorientation, slurred or rambling speech, irrational behavior, and extreme fatigue and lethargy may occur. Neuroglycopenia is usually the cause of physical injuries and accidents that occur during hypoglycemic episodes. Most patients with deficits in epinephrine will experience only signs of neuroglycopenia. Patients and their family/caregivers should be taught the early warning signs of neuroglycopenic symptoms (e.g., slow thinking, blurred vision, slurred speech, numbness, trouble concentrating, dizziness, fatigue, and sleepiness).[83] Patients should be encouraged to keep a symptom diary and record their symptoms whenever they measure their blood glucose.

Hypoglycemic episodes are usually caused by excess in blood glucose lowering medications (e.g., insulin and insulin secretagogues), physical activity, or inadequate carbohydrate intake. It is important to carefully examine the individual's insulin regimen. Hypoglycemia frequently occurs when insulin action is peaking. Individuals should also check their blood glucose levels after exercising. Alcohol consumption in the absence of food intake may also result in hypoglycemia.[84]

Sulfonylureas used concomitantly with sulfa-type antibiotics (e.g., trimethoprim–sulfamethoxazole) can cause severe and refractory hypoglycemia. This interaction results from displacement of the sulfonylureas from their protein binding sites by sulfa-type antibiotics. Patients with DM who are taking a sulfonylurea must be educated to inform their healthcare provider if a sulfa-type antibiotic is also prescribed. Individuals with DM should be taught how to avoid a hypoglycemic episode. Consumption of a high-fat meal may slow gastric emptying and the absorption of carbohydrates. Therefore, eating a high-fat, low-carbohydrate meal after an insulin injection may result in hypoglycemia.

15/15 Rule

Individuals should be taught that all blood glucose levels below 70 mg/dL (3.9 mmol/L) should be treated, even in the absence of symptoms. Patients should eat or drink 10–15 g of glucose-containing or carbohydrate-containing food or beverage, which should increase blood glucose levels by 30–45 mg/dL. Foods and beverages, which contain 15 g of carbohydrate, include 4 ounces of fruit juice, 4 ounces of nondiet soda, 8–10 Life Savers, or 3–4 glucose tablets. Avoid drinks and foods that are high in fat (e.g., chocolate or whole milk) that may slow absorption of carbohydrates and take longer to raise blood glucose levels. Ingesting protein such as meat will not treat hypoglycemia and will not raise blood glucose levels. Test blood glucose levels 15–20 minutes later. If blood glucose levels are still low, repeat the treatment. Patients should be informed not to miss meals and to have a bedtime snack if blood glucose levels are <120 mg/dL (6.7 mmol/L).[83,84]

Other Laboratory Tests Used in the Management of Diabetes Mellitus

The leading cause of death in patients with DM is cardiovascular disease (CVD). Control of hypertension and dyslipidemia is necessary to decrease the risk of macrovascular complications. Under the new American College of Cardiology and American Heart Association lipid guidelines, patients should be placed on statin medications based on risk stratification and treated with varying intensity statin dosing regimens.[85] The new 2015 ADA standards recommends the following for patients with diabetes[86]:

- All patients with CVD should receive high intensity statin therapy.
- Patients <40 years of age with additional CVD risk factors should receive moderate-to-high intensity statin therapy.
- Patients >40 years of age without additional CVD risk factors should receive moderate-intensity statin therapy.
- Patients 40–75 years of age with additional CVD risk factors should receive high-intensity statin therapy.
- Patients >75 years of age with additional CVD risk factors should receive moderate or high-intensity statin therapy.
- Statin therapy should be adjusted based on individual patient response to medication including side effects, tolerability, and low-density lipoprotein cholesterol levels.[85,86]

Both systolic and diastolic hypertension increase the risk of microvascular and macrovascular complications in patients with DM. Aggressive treatment of hypertension can attenuate the progression of complications. The ADA currently recommends a goal blood pressure (BP) of <140/90 mm Hg, but a lower systolic target of <130 mm Hg may be appropriate in younger patients if it can be obtained without exposing the patient to adverse effects associated with treatment.[86]

Urinalysis for detection of proteinuria should be obtained in patients with DM on a yearly basis. This should begin at the time of diagnosis in patients with type 2 DM and five years after diagnosis in patients with type 1 DM.[87] A quantitative test for urine protein should follow a positive result on urinalysis. If urinalysis is negative for proteinuria, testing for increased albumin excretion (previously termed *microalbuminuria*) should be obtained. Increased albumin excretion indicates glomerular damage and is predictive of clinical nephropathy.[86]

Three methods are available to screen for increased albumin excretion. One method is measurement of the urine albumin to creatinine ratio in a spot urine sample. This method is convenient in the clinical setting, as it requires only one urine sample. A morning sample is preferred to take into account the diurnal variation of albumin excretion. A second method is a 24-hour urine collection for determination of albumin excretion. This method may be tedious and accuracy relies on proper collection techniques. An advantage of this method is that renal function can simultaneously be quantified. A third alternative method to the 24-hour collection is a timed urine collection for albumin. Moderately increased albuminuria is defined as a urinary albumin excretion of 30–299 mcg/mL on a spot urine sample, 30–299 mg/24 hr on a 24-hour urine collection or 20–199 mcg/min on a timed urine collection. Transient rises in albumin excretion can be associated with exercise, hyperglycemia, hypertension, urinary tract infection, heart failure, and fever. Therefore, if any of these conditions are present, they may result in false positives on screening tests. Variability exists in the excretion of albumin; thus, moderately increased albuminuria must be confirmed in two repeated tests in a three- to six-month period. Two of three positive screening tests for moderately increased albuminuria confirm the diagnosis.[87-89]

Based on results of landmark studies, the ADA recommends angiotension-converting enzyme (ACE) inhibitors or angiotension receptor blockers (ARBs) for the treatment of both moderately increased albuminuria and severely increased albuminuria (previously termed *macroalbuminuria* and defined as a urinary albumin excretion >300 mg/24 hr on a 24-hour urine collection). If one class is not tolerated, the other should be substituted. Patients with DM and hypertension should be treated with pharmacologic therapy regimen that includes either an ACE inhibitor or an ARB. Although ARBs have been shown to delay the progression of nephropathy in patients with type 2 DM, hypertension, moderately increased albuminuria, and renal insufficiency, ACE inhibitors are the initial agents of choice in patients with type 1 DM with hypertension and any degree of albuminuria. Thiazide diuretics, β-blockers, or calcium channel blockers should be used as an add-on agent to further decrease BP.[87-90]

THYROID

Anatomy and Physiology

The *thyroid* gland is a butterfly-shaped organ composed of two connecting lobes that span the trachea. The thyroid produces the hormones thyroxine (T_4) and triiodothyronine (T_3). Approximately 80 and 30 mcg of T_4 and T_3, respectively, are produced daily in normal adults. Although T_4 is produced solely by the thyroid gland, only about 20–25% of T_3 is directly secreted by this gland. Approximately 80% of T_3 is formed by hepatic and renal deiodination of T_4.

T_4 has a longer half-life than T_3, approximately seven days versus one day, respectively. At the cellular level, however, T_3 is three to four times more active physiologically than T_4.[91] When the conversion of T_4 to T_3 is impaired, a stereoisomer of T_3, known as *reverse T_3*, is produced; reverse T_3 has no known biological effect.

Thyroid hormones have many biological effects, both at the molecular level and on specific organ systems. These hormones stimulate the basal metabolic rate and can affect protein, carbohydrate, and lipid metabolism. They are also essential for normal growth and development. Thyroid hormones act to

- stimulate neural and skeletal development during fetal life
- stimulate oxygen consumption at rest
- stimulate bone turnover by increasing bone formation and resorption
- promote the conversion of carotene to vitamin A
- promote chronotropic and inotropic effects on the heart
- increase the number of catecholamine receptors in heart muscle cells
- increase basal body temperature
- increase the production of RBCs
- increase the metabolism and clearance of steroid hormones
- alter the metabolism of carbohydrates, fats, and protein
- control the normal hypoxic and hypercapnic respiratory drives

The synthesis of thyroid hormones depends on iodine and the amino acid tyrosine. The thyroid gland, using an energy-requiring process, transports dietary iodide (I^-) from the circulation into the thyroid follicular cell. Iodide is oxidized to iodine (I_2), and then combined with tyrosyl residues within the thyroglobulin molecule to form thyroid hormones (iodothyronine). Thus, thyroid hormones are formed and stored within the thyroglobulin protein for release into the circulation.[91-95]

Both T_4 and T_3 circulate in human serum bound to three proteins: thyroxine-binding globulin (TBG), transthyretin, previously known as *thyroid-binding prealbumin*, and albumin. Of the three proteins, 80% of T_4 and T_3 is bound to TBG. Only 0.02% of T_4 and 0.2% of T_3 circulate unbound, free to diffuse into tissues.[96] The “free” fraction is the physiologically active component. Total and free hormones exist in an equilibrium state in which the protein-bound fraction serves as a reservoir for making the free fraction available to tissues.[97]

Thyroid hormone secretion is regulated by a feedback mechanism involving the hypothalamus, anterior pituitary, and thyroid gland itself (**Figure 10-4**). The release of T_4 and T_3 from the thyroid gland is regulated by *thyrotropin*, also called *thyroid-stimulating hormone* (TSH), which is secreted by the anterior pituitary. The intrathyroidal iodine concentration also influences thyroid gland activity, and TSH secretion primarily is regulated by a dual negative feedback mechanism:

Thyrotropin-releasing hormone (TRH) or protirelin is released by the hypothalamus, which stimulates the synthesis and release of TSH from the pituitary gland. Basal TSH concentrations in persons with normal thyroid function are about 0.3–5 milliunits/L. The inverse relationship between TSH and free T_4 is logarithmic. A 50% decrease in free T_4 concentrations leads to a 50-fold increase in TSH concentrations and

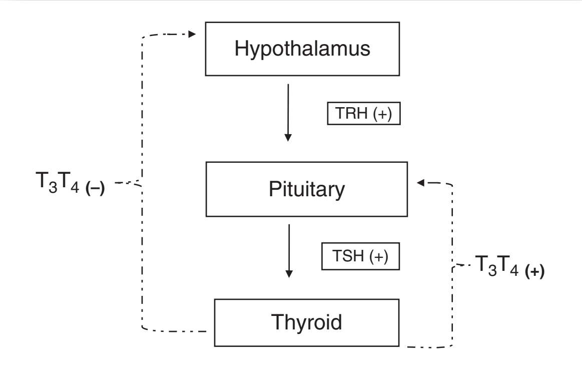


FIGURE 10-4. The hypothalamic-pituitary-thyroid axis.

vice versa.[98] Unbound T_4 and T_3 (mainly the concentration of intracellular T_3 in the pituitary) directly inhibit pituitary TSH secretion. Consequently, increased concentrations of free thyroid hormones cause decreased TSH secretion, and decreased concentrations of T_4 and T_3 cause increased TSH secretion.[98]

Prolonged exposure to cold and acute psychosis may activate the hypothalamic-pituitary-thyroid axis, whereas severe stress may inhibit it. Although TRH stimulates pituitary TSH release, somatostatin corticosteroids and dopamine inhibit it. Small amounts of iodide are needed for T_4 and T_3 production, but large amounts inhibit their production and release. Most recent evidence based on the most sensitive assays suggests that no physiologically relevant change in serum TSH concentrations occurs in relation to age.[97]

Thyroid Disorders

Patients with a normally functioning thyroid gland are said to be in a *euthyroid* state. When this state is disrupted, thyroid disease may result. It occurs four times more often in women than in men and may occur at any age, but it peaks between the third and sixth decades of life. A family history of this disease often is present, especially for the autoimmune thyroid diseases. Diseases of the thyroid usually involve an alteration in the quantity or quality of thyroid hormone secretion and may manifest as *hypothyroidism* or *hyperthyroidism*. In addition to the signs and symptoms discussed below, thyroid disease may produce an enlargement of the thyroid gland known as *goiter*.

Hypothyroidism

Hypothyroidism results from a deficiency of thyroid hormone production, causing body metabolism to slow down. This condition affects about 2% of women and 0.2% of men and the incidence increases with age. Symptoms include lethargy; constipation; dry, coarse skin and hair; paresthesias and slowed deep tendon reflexes; facial puffiness; cold intolerance; decreased sweating; impaired memory, confusion, and dementia; slow speech and motor activity; and anemia and growth retardation in children. Interestingly, these typical signs and symptoms have been observed in as few as 25% of elderly hypothyroid patients.[100,101]

Hypothyroidism is usually caused by one of three mechanisms. Primary hypothyroidism is a failure of the thyroid to produce thyroid hormone; secondary hypothyroidism is failure of the anterior pituitary to secrete TSH; and tertiary hypothyroidism is failure of the hypothalamus to produce TRH. Most patients with symptomatic primary hypothyroidism have TSH concentrations >20 milliunits/L. Patients with mild signs or symptoms (usually not the reason for the visit to the doctor) have TSH values of 10–20 milliunits/L. Patients with secondary and tertiary hypothyroidism may have a low or normal TSH, but other hormones (e.g., prolactin, cortisol, and gonadotropin) can be measured to confirm pituitary insufficiency. **Table 10-5** outlines the numerous etiologies of hypothyroidism.

TABLE 10-5. Classification of Hypothyroidism by Etiology[91,102,103]

Primary
Iodine deficiency
Excessive iodide intake (e.g., kelp and contrast dyes)
Thyroid ablation: surgical removal of the thyroid, post I^{131} (radioactive Iodine I^{131}) treatment of thyrotoxicosis, radiation of neoplasm
Hashimoto (autoimmune) thyroiditis
Subacute thyroiditis
Genetic abnormalities of thyroid hormone synthesis
Medications: propylthiouracil, methimazole, thiocyanate, lithium, amiodarone
Food: excessive intake of goitrogenic foods (e.g., cabbage and turnips)
Secondary
Hypopituitarism: adenoma, ablative therapy, pituitary
destruction, sarcoidosis hypothalamic dysfunction
Other
Abnormalities of T_4 receptor

T_4 = thyroxine.

Thyrotoxicosis

Thyrotoxicosis results when excessive amounts of thyroid hormones are circulating and is usually due to hyperactivity of the thyroid gland (hyperthyroidism). Signs and symptoms include nervousness; fatigue; weight loss; heat intolerance; increased sweating; tachycardia or atrial fibrillation; muscle atrophy; warm, moist skin; and, in some patients, exophthalmos. These signs and symptoms occur much less frequently in the elderly, except for atrial fibrillation, which occurs three times more often.[99] **Table 10-6** summarizes the specific causes of hyperthyroidism.

TABLE 10-6. Classification of Hyperthyroidism by Etiology[91,100,102,103]

Overproduction of thyroid hormone
Graves disease[a]
TSH-secreting pituitary adenomas
Hydatidiform moles/choriocarcinomas[b]
Multinodular goiter
“Leaking” thyroid hormone due to thyroid destruction
Lymphocytic thyroiditis
Granulomatous thyroiditis
Subacute thyroiditis
Radiation
Medications: thyroid-replacement drugs (excessive), amiodarone, iodinated radiocontrast agents, iodine excess, kelp[c]
Ovarian teratomas with thyroid elements
Metastatic thyroid carcinoma

TSH = thyroid-stimulating hormone.

[a] Most frequent cause. The mechanism is production of thyroid-stimulating antibodies; usually associated with diffuse goiter and ophthalmopathy.

[b] Tumor production of chorionic gonadotropin, which stimulates the thyroid.

[c] Patients at risk of hyperthyroidism from these agents usually have some degree of thyroid autonomy.

Nonthyroid Laboratory Tests in Patients with Thyroid Disease

Both hypothyroidism and hyperthyroidism may cause pathophysiology outside the thyroid gland. **Table 10-7** lists nonthyroid laboratory tests that may indicate a thyroid disorder. The influence on these tests reflects the widespread effects of thyroid hormones on peripheral tissues. Findings from these tests cannot be used alone to diagnose a thyroid disorder. However, they may support a diagnosis of thyroid dysfunction when used with specific thyroid function tests and the patient's presenting signs and symptoms.

Thyroid Function Tests

Tests more specific for thyroid status or function can be categorized as those that (1) measure the concentration of products secreted by the thyroid gland, (2) evaluate the integrity of the hypothalamic-pituitary-thyroid axis, (3) assess intrinsic thyroid gland function, and (4) detect antibodies to thyroid tissue.[104] Tests that directly or indirectly measure the concentrations of T_4 and T_3 include the following:

- Free T_4
- Total serum T_4
- Serum T_3 resin uptake
- Free T_4 index
- Total serum T_3

TABLE 10-7. Nonthyroid Laboratory Tests Consistent with Thyroid Disorders[91,94,102,103]

HYPOTHYROIDISM	HYPERTHYROIDISM
Decreased	
Hgb/Hct[a]	Granulocytes
Serum glucose	Serum cholesterol
Serum sodium	Serum triglycerides
Urinary excretion of 17-hydroxysteroids	
Urinary excretion of 17-ketosteroids	
Increased	
AST/SGOT	Alkaline phosphatase
Capillary fragility	Lymphocytes
Cerebrospinal fluid protein	Serum ferritin
LDH	Urinary calcium excretion
pCO_2	
Serum carotene	
Serum cholesterol	
CPK	
Serum prolactin	
Serum triglycerides	

AST = aspartate aminotransferase; CPK = serum creatine phosphokinase; Hct = hematocrit; Hgb = hemoglobin; LDH = lactate dehydrogenase; pCO_2 = partial pressure of carbon dioxide; SGOT = serum glutamic-oxaloacetic transaminase.

[a] Associated with normocytic and macrocytic anemias.

The integrity of the hypothalamic-pituitary-thyroid axis is assessed by measuring TSH and TRH. A radioactive iodine uptake test assesses intrinsic thyroid gland function, and an antithyroid antibodies test detects antibodies to thyroid tissue.

Free T_4

Normal range: 0.9–2.3 ng/dL (11.6–29.6 pmol/L)

This test measures the unbound T_4 in the serum and is the most accurate reflection of thyrometabolic status. The low concentration of *free T_4* in the serum (<1% of total T_4) makes accurate measurement a difficult and laborious process. Therefore, free T_4 is assayed primarily when TBG alterations or nonthyroidal illnesses confound interpretation of conventional tests (**Table 10-8** and **Table 10-9**).

Several methods can determine free T_4. Some methods perform well only in otherwise healthy hypothyroid and hyperthyroid patients and in euthyroid patients with mild abnormalities of TBG. However, in patients with severe

TABLE 10-8. Free T_4 and TSH in Thyroidal and Nonthyroidal Disorders

DIAGNOSIS	FREE T_4 INDEX OR DIRECT EQUILIBRIUM DIALYSIS FREE T_4	TSH (milliunits)
Hypothyroidism		
Primary Normal	↓	↑
On dopamine or glucocorticoids	↓	↓
Secondary or tertiary—functional hypopituitarism	↓	↓
Recent thyroid supplement withdrawal	↓	<0.1
Recently treated hyperthyroidism	↓	<0.1
Hyperthyroidism	↑	<0.1[a]
With severe nonthyroidal illness	↓/WNL/↑[b]	<0.1[a]
Euthyroid states	WNL	WNL
Low total T_4 of nonthyroidal illness	↓/WNL/↑[c]	WNL/↑
After T_3 therapy	↓	WNL/↓
After T_4 therapy	WNL	WNL
High total T_4 of nonthyroidal illness	↑	WNL/↑
High total T_4 from amiodarone or iodinated contrast media	↑	↑
Decreased T_4-binding proteins	↑/WNL[d]	WNL

↑ = increased; ↓ = decreased; T_3 = triiodothyronine; T_4 = thyroxine; TSH = thyroid-stimulating hormone; WNL = within normal limits.

[a] Usually absent TSH response to TRH; also may be normal with hyperthyroidism from TSH-secreting tumors.

[b] Normal or low using free T_4 index estimation; increased using the direct equilibrium dialysis free T_4 assay.

[c] Decreased using free T_4 index estimation; normal to high using the direct equilibrium dialysis free T_4 assay.

[d] Decreased using free T_4 index estimation; normal using the direct equilibrium dialysis free T_4 assay.

TABLE 10-9. Performance and Availability of Free T_4 Methods

ASSAY	% OF EUTHYROID PATIENTS WITH SEVERE TBG DEPRESSION OR SEVERE NONTHYROIDAL ILLNESS IN WHICH ASSAY UNDERESTIMATES FREE T_4	COMMENTS
Free T_4 index[a] or single-step	50–80%[106]	Available in most clinical laboratory tests
Immunoextraction or RIA[b]	10–30%[93]	Available in some clinical laboratory tests
Direct equilibrium dialysis[c]	0–5%[107,d]	Available in reference laboratory tests and large medical center laboratory tests; gold standard
Ultrafiltration[c]	0–5%[107]	Available only in research laboratory tests

RIA = radioimmunoassay; T_4 = thyroxine.

[a] Corrects total T_4 values using an assessment of T_4-binding proteins.

[b] Uses a T_4 analog or two-step-back titration with solid-phase T_4 antibody but does not use membranes to separate free from bound hormone.

[c] Uses minimally diluted serum that separates free T_4 from bound T_4 using a semipermeable membrane.

[d] May be underestimated in about 25% of patients on dopamine.[108] A decreased direct equilibrium dialysis free T_4 with an elevated TSH is diagnostic of primary hypothyroidism, even in patients with severely depressed TBG. Conversely, an increased direct equilibrium dialysis free T_4 with a TSH of <0.10 milliunit/L is consistent with nonpituitary hyperthyroidism.[53] Decreased direct equilibrium dialysis free T_4 with normal or decreased TSH concentrations may be seen in patients on T_3 therapy (Table 10-8). Although free T_4 assays are becoming widely available (Table 10-12), most clinicians initially rely on the traditional total serum T_4 measurement by RIA.

alterations of T_4 binding to carrier proteins (e.g., severe nonthyroidal illness), only the direct equilibrium dialysis method maintains accuracy (Table 10-9).[106]

Total Serum T_4

Normal range: 5.5–12.5 mcg/dL (71–161 nmol/L)

Although ultrasensitive TSH and free T_4 assays are gradually supplanting RIA methodology, total serum T_4 by RIA is the standard initial screening test to assess thyroid function because of its wide availability and quick turnaround time. In most patients, the *total serum T_4* level is a sensitive test for the functional status of the thyroid gland. It is high in 90% of hyperthyroid patients and low in 85% of hypothyroid patients. This test measures both bound and free T_4 and is, therefore, influenced by any alteration in the concentration or binding affinity of thyroid-binding protein.

Conditions that increase or decrease thyroid-binding protein result in an increased or decreased total serum T_4, respectively, but do not affect the amount of metabolically active free T_4 in the circulation. Therefore, thyrometabolic status may not always be truly represented by the results. To circumvent this problem, most clinicians concomitantly obtain the T_3 resin uptake test (discussed later) so they can factor out this interference. **Table 10-10** lists factors that alter thyroid-binding protein.

TABLE 10-10. Factors Altering Thyroid-Binding Protein[91,94,97,99,102]

FACTORS THAT INCREASE THYROID-BINDING PROTEIN	FACTORS THAT DECREASE THYROID-BINDING PROTEIN
Acute infectious hepatitis	Acromegaly
Acute intermittent porphyria	Androgen therapy
Chronic active hepatitis	L-asparaginase
Clofibrate	Cirrhosis
Estrogen-containing oral contraceptives	Danazol
Estrogen-producing tumors	Salsalate
Estrogen therapy	Genetic deficiency of TBG
5-fluorouracil	Glucocorticoid therapy (high dose)
Genetic excess of total binding protein	Furosemide (high dose)
Heroin	Hypoproteinemia
Methadone maintenance	Malnutrition
Perphenazine	Nephrotic syndrome
Pregnancy	Phenytoin
Tamoxifen	Salicylates
	Testosterone-producing tumors

TBG = thyroxine-binding globulin.

Increased total serum T4. An increased total serum T_4 may indicate hyperthyroidism, elevated concentrations of thyroid-binding proteins, or nonthyroid illness. Total serum T_4 elevations have been noted in patients, particularly the elderly, with relatively minor illnesses. These transient elevations may be due to increased TSH secretion stimulated by a low T_3 concentration. Similarly, up to 20% of all patients admitted to psychiatric hospitals have had transient total serum T_4 elevations on admission.[71,83] Thus, the differential diagnosis for a patient with this elevation must include nonthyroid illness versus hyperthyroidism if other signs and symptoms of thyroid disease are absent or inconsistent.

Decreased total serum T4. A decreased total serum T_4 may indicate hypothyroidism, decreased concentrations of thyroid-binding proteins, or *nonthyroid illness* (also called *euthyroid sick syndrome*). Nonthyroid illness may lower the total serum T_4 concentration with no change in thyrometabolic status. Typically in this syndrome, total serum T_4 is decreased (or normal), total serum T_3 is decreased, reverse T_3 is increased, and TSH is decreased (or normal). Neoplastic disease, DM, burns, trauma, liver disease, renal failure, prolonged infections, and CVD are nonthyroid illnesses that can lower total serum T_4 concentrations.

TABLE 10-11. Medications That Cause a True Alteration in Total Serum T_4 and Free T_4 Measurements[a]

MECHANISM	INCREASE TOTAL SERUM T_4 AND FREE T_4	DECREASE TOTAL SERUM T_4 AND FREE T_4
Interference in central regulation of TSH secretion at hypothalamic-pituitary level	Amphetamines	Glucocorticoids (acutely) Octreotide Dobutamine Dopamine
Interference with thyroid hormone synthesis or release from thyroid gland	Amiodarone,[b] lithium[b]	Aminoglutethimide, amiodarone,[b] 6-mercaptopurine, sulfonamides iodides[b] Thionamides lithium[b]
Altered thyroid hormone metabolism	Amiodarone, iopanoic acid, ipodate, propranolol (high dose)	Phenobarbital Carbamazepine Rifampin Phenytoin
Inhibition of GI absorption of exogenous thyroid hormone		Antacids, orlistat cholestyramine, colestipol, iron, sodium polystyrene sulfonate, soybean flour (infant formulas), sucralfate Omeprazole lansoprazole

GI = gastrointestinal; T_3 = triiodothyronine; T_4 = thyroxine; TSH = thyroid-stimulating hormone.

[a] In true alterations, the concentration change is not due to assay interference or alteration in thyroid-binding proteins. As noted in Table 10-12, iodides can significantly alter thyroid status. They have the potential to inhibit thyroid hormone release and to impair the organification of iodine. In healthy individuals, this effect lasts only one to two weeks. However, individuals with subclinical hypothyroid disease may develop clinical hypothyroidism after treatment with iodides. Iodide-induced hypothyroidism has also been noted in patients with cystic fibrosis and emphysema.[96]

[b] May increase or decrease total serum T_4 and free T_4.

Source: Compiled, in part, from references 91, 94, 96, 98, and 101.

Several mechanisms probably contribute to this low T_4 state. Diminished T_4 in nonthyroid illness may be due to low TBG concentrations caused by protease cleavage at inflammatory sites during acute inflammatory illness. In some, but not all patients with chronic illness, a desialylated form of TBG is synthesized by the liver, which has one tenth the binding capacity of that of normal TBG. This results in a fall in the circulating levels of total thyroid hormone as a consequence of the diminished thyroid hormone-binding capacity. In addition, peripheral deiodination of T_4 to T_3 is impaired because of diminished activity of type I deiodinase enzyme. Diminished enzyme activity accounts for decreased deiodination of T_4 to T_3 and an increase in the production of reverse T_3.

In general, a correlation exists between the degree of total serum T_4 depression and the prognosis of the illness (i.e., the lower the total serum T_4, the poorer the disease outcome). Because severely ill patients may appear to be hypothyroid, it is important to differentiate between patients with serious nonthyroid illnesses and those who are truly hypothyroid.[82,85]

TABLE 10-12. Iodine-Containing Compounds That May Influence Thyroid Status

Oral radiopaque agents
Diatrizoate
Iocetamic acid
Iopanoic acid
Ipodate
Tyropanoate
Expectorants
Iodinated glycerol[a]
Potassium iodide solution
SSKI
Parenteral radiopaque agents
Diatrizoate meglumine
Iodamide meglumine
Iopamidol
Iothalamate meglumine
Metrizamide
Miscellaneous compounds
Amiodarone
Kelp-containing nutritional supplements

SSKI = supersaturated potassium iodide.

[a] No longer available; most products reformulated with guaifenesin.

Source: Compiled, in part, from references 97 and 102.

After recovery from an nonthyroid illness, thyroid function test result abnormalities should be completely reversible.

Drugs causing true alterations in total serum thyroxine. Medications can cause a true alteration in total serum T_4 and a corresponding change in free T_4 concentrations (**Tables 10-11 and 10-12**). In such cases, the total serum T_4 (and free T_4) result remains a true reflection of thyrometabolic status. High-dose salicylates and phenytoin also may lower total serum T_4 significantly via decreased protein binding in vivo. Salicylates inhibit binding of T_4 and T_3 to TBG. An initial increase in serum free T_4 is followed by return of free T_4 to normal levels with sustained therapeutic serum salicylate concentrations, although total T_4 levels may decrease by as much as 30%. Phenytoin displaces T_4 and T_3 from serum binding proteins resulting in an initial increase in free T_4 and T_3, and a decrease in total T_4 levels.[110]

Iodides may also *increase* thyroid function. A previously euthyroid patient may develop thyrotoxicosis from exposure to increased quantities of iodine. Supplemental iodine causes autonomously functioning thyroid tissue to produce and secrete thyroid hormones, leading to a significant increase in T_4 and T_3 concentrations. This phenomenon commonly occurs during therapeutic iodine replacement in patients who live in areas of endemic iodine deficiency.

Similarly, patients with underlying goiter who live in iodine-sufficient areas may develop hyperthyroidism when given pharmacological doses of iodide. The heavily iodinated antiarrhythmic medication amiodarone may induce hyperthyroidism (1–5% of patients) as well as hypothyroidism (6–10% of patients).[110,111] Propylthiouracil and methimazole are used in hyperthyroid patients to decrease hormone concentrations. Both T_4 and T_3 concentrations decrease more rapidly with methimazole than propylthiouracil.[112]

Serum T_3 Resin Uptake

Normal range: 25–38%

The *serum T_3 resin uptake* test indirectly estimates the number of binding sites on thyroid-binding protein occupied by T_3. This result is also referred to as the *thyroid hormone-binding ratio.* The T_3 resin uptake is usually low when the concentration of thyroid-binding proteins is high.[102,103]

In this test, radiolabeled T_3 is added to a specimen that contains endogenous hormone. An aliquot of this mixture is then added to a resin that competes with endogenous thyroid-binding proteins for the free hormone. Radiolabeled T_3 binds to any free endogenous thyroid-binding protein; at the saturation point, the remainder binds to the resin. The amount of thyroid-binding protein can be estimated from the amount of radiolabeled T_3 taken up by the resin. The T_3 resin uptake result is expressed as a percentage of the total radiolabeled T_3 that binds to the resin. The T_3 resin uptake can verify the clinical significance of measured total serum T_4 and T_3 concentrations because it is an indicator of thyroid-binding protein-induced alterations of these measurements.[102,103]

Elevated T_3 resin uptake concentrations are consistent with hyperthyroidism, while decreased concentrations are consistent with hypothyroidism. However, this test is never used alone for diagnosis. The T_3 resin uptake is low in hypothyroidism because of the increased availability of binding sites on the TBG. However, in nonthyroidal illnesses with a low T_4, the T_3 resin uptake is elevated. Therefore, the test may be used to differentiate between true hypothyroidism and a low T_4 state caused by nonthyroid illness.[102,103]

All of the disease states and medications listed in **Table 10-13** can influence thyroid-binding protein and, consequently, alter T_3 resin uptake results. Radioactive substances taken by the patient also will interfere with this test. In practice, the T_3 resin uptake test is used only to calculate the free T_4 index.

Free T_4 Index

Normal range: 1–4 units

The *free T_4 index* is the product of total serum T_4 multiplied by the percentage of T_3 resin uptake:

$$\text{free } T_4 \text{ index} = \text{total serum } T_4 \text{ (mcg/dL)} \times T_3 \text{ resin uptake (\%)}$$

The free T_4 index adjusts for the effects of alterations in thyroid-binding protein on the total serum T_4 assay. The index is high in hyperthyroidism and low in hypothyroidism. Patients taking phenytoin or salicylates have low total serum T_4 and high T_3 resin uptake with a normal free T_4 index. Pregnant patients have high total serum T_4 and low T_3 resin uptake with a normal free T_4 index. Patients taking therapeutic doses of levothyroxine may have a high free T_4 index because total serum T_4 and T_3 resin uptake are high. In addition to affecting total serum T_4 and free T_4 (Table 10-13), propranolol and nadolol block the conversion of T_4 to T_3, which may cause mild elevations in the free T_4 index.[101,109]

Total Serum T_3

Normal range: 80–200 ng/dL (1.2–3.1 nmol/L)

Using RIA highly active thyroid hormone T_3 is measured. Like T_4, almost all of T_3 is protein bound. Therefore, any alteration in thyroid-binding protein influences this measurement. As with the total serum T_4 test, changes in thyroid-binding protein increase or decrease total serum T_3 but do not affect the metabolically active free T_3 in the circulation. Therefore, the patient's thyrometabolic status remains unchanged.

Total serum T_3 is primarily used as an indicator of hyperthyroidism. This measurement is usually made to detect T_3 toxicosis when T_3, but not T_4, is elevated. Generally, the serum T_3 assay is not a reliable indicator of hypothyroidism because of the lack of reliability of the assay in the low to normal range. Drugs that affect T_4 concentrations (Tables 10-11 and 10-13)

TABLE 10-13. Test Results Seen in Common Thyroid Disorders and Drug Effects on Test Results[94,105,109,125,126]

DISEASE	TOTAL SERUM T_4	TOTAL SERUM T_3	T_3 RESIN UPTAKE	FREE T_4 INDEX	RAIU	TSH	COMMENT
Hypothyroidism	↓	↓	↓	↓	↓	↑/↓ [a]	
Hyperthyroidism	↑	↑	↑	↑	↑	↓	
T_3 thyrotoxicosis	No change	↑	No change	No change	No change	↓	T_3 resin uptake may be slightly increased
Euthyroid sick	No change/↓	↓	↑	Variable	No change	No change syndrome	
Corticosteroids	↓	No change/↓	↑	No change	↓	No change/↓	
Phenytoin/aspirin	↓	↓	↑	No change	↓	No change	Large salicylate dose
Radiopaque media	No change/↑	No change/↓	No change	No change/↑	↓	No change	

↑ = increased; ↓ = decreased; RAIU = radioactive iodine uptake test; T_3 = triiodothyronine; T_4 = thyroxine; TSH = thyroid-stimulating hormone.

[a] Increased TSH diagnostic of primary hypothyroidism. TSH is decreased in secondary and tertiary types.

have a corresponding effect on T_3 concentrations. Additionally, propranolol, propylthiouracil, and glucocorticoids inhibit the peripheral conversion of T_4 to T_3 and cause a decreased T_3 concentration (T_4 usually stays normal).[112]

Total serum T_3 concentrations can be low in euthyroid patients with conditions (e.g., malnutrition, cirrhosis, and uremia) in which the conversion of T_4 to T_3 is suppressed. T_3 is low in only half of hypothyroid patients because these patients tend to produce relatively more T_3 than T_4. A patient with a normal total serum T_4, a low T_3, and a high reverse T_3 has euthyroid sick syndrome.

Thyroid-Stimulating Hormone

Normal range: 0.5–5.0 milliunits/L

Thyroid-stimulating hormone (TSH) is a glycoprotein with two subunits, alpha (α) and beta (β). The α subunit is similar to those of other hormones secreted from the anterior pituitary: follicle-stimulating hormone, human chorionic gonadotropin (hCG), and luteinizing hormone. The β subunit of TSH is unique and renders its specific physiological properties.[102,103]

Although the older "first-generation" TSH assays have been useful in diagnosing primary hypothyroidism, they have not been useful in diagnosing hyperthyroidism. Almost all patients with symptomatic primary hypothyroidism have TSH concentrations >20 milliunits/L; those with mild signs or symptoms have TSH values of 10–20 milliunits/L. Often, TSH concentrations become elevated before T_4 concentrations decline. All assays can accurately measure high concentrations of TSH.[102,103]

The first-generation TSH assays, however, cannot distinguish low–normal from abnormally low values because their lower limit of detection is 0.5 milliunits/L, while the lower limit of basal TSH is 0.2–0.3 milliunits/L in most euthyroid persons. This distinction can usually be ascertained with the second-generation assays, which can accurately measure TSH concentrations as low as 0.05 milliunits/L. Occasionally, some euthyroid patients have levels of 0.05–0.5 milliunits/L. Therefore, supersensitive, third-generation and fourth-generation assays have been developed; they can detect TSH concentrations as low as 0.005 milliunits/L and 0.004 milliunits/L, respectively. Although third-generation assays are usually not required to make or confirm this diagnosis, they provide a wider margin of tolerance so that discrimination at 0.1 milliunit/L can be ensured even when the assay is not performing optimally.[109] Concentrations below 0.05 milliunits/L are almost always diagnostic of primary hyperthyroidism in patients younger than 70 years.[102,103,112]

Use in therapy. In patients with primary hypothyroidism, TSH concentrations are also used to adjust the dosage of levothyroxine replacement therapy. In addition to achieving a clinical euthyroid state, the goal should be to lower TSH into the midnormal range (**Minicase 3**). Although TSH concentrations reflect long-term thyroid status, serum T_4 concentrations reflect acute changes. Patients with long-standing hypothyroidism often notice an improvement in well-being two to three weeks after starting therapy. Significant improvements in heart rate, weight, and puffiness are seen early in therapy, but hoarseness, anemia, and skin/hair changes may take many months to resolve.[115]

Unless undesirable changes in signs or symptoms occur, it is rational to wait at least six to eight weeks after starting or changing therapy to repeat TSH and T_4 concentrations to refine dosing.[93,115] The hypothalamic–pituitary axis requires this time to respond fully to changes in circulating thyroid hormone concentrations. This slow readjustment can be exploited elsewhere. One study found that >50% of TSH elevations in patients being treated with levothyroxine were attributed to noncompliance; with counseling alone, TSH normalized on subsequent visits.[116] Noncompliant hypothyroid patients who take their levothyroxine pills only before being tested may have elevated TSH concentrations despite a normal T_4 concentration.[108]

Because of slow axis readjustment, patients given antithyroid drugs (e.g., methimazole) may maintain low TSH concentrations for two to three months after T_4 and T_3 concentrations have returned to normal. Single, daily doses of 10–20 mg of methimazole usually lead to euthyroidism within several weeks.[117] Treatment should not be adjusted too early using (low) TSH concentrations alone. However, the dose should be reduced within the first eight weeks if TSH concentrations become elevated.

Patients with thyroid cancer are often treated with TSH suppressive therapy, usually levothyroxine. The therapeutic endpoint is a basal TSH concentration of about 0.1 milliunit/L.[118] Some clinicians suggest more complete suppression with TSH concentrations <0.005 milliunit/L, while others think that it leads to toxic effects of overreplacement (e.g., accelerated bone loss) (**Minicase 4**).[108,116]

Potential misinterpretation and drug interference. Some TSH assays may yield falsely high results whenever hCG concentrations are high (i.e., pregnancy) due to the similarity in structure of these two proteins. Most patients who have secondary or tertiary hypothyroidism have low or normal TSH concentrations. In patients with nonthyroid illness, TSH may be suppressed by factors other than thyroid hyperfunction. As mentioned previously, the TSH concentration typically is normal in patients with euthyroid sick syndrome.

Thyroid function tests are known to be altered in depressed patients. With the advent of the third-generation TSH assays, investigators hoped that TSH concentrations could help to determine various types of depression and response to therapies. Unfortunately, TSH has not proven useful in this way.[120] Because endogenous dopamine inhibits the stimulatory effects of TRH, any drug with dopaminergic activity can inhibit TSH secretion. Therefore, levodopa, glucocorticoids, bromocriptine, and dopamine are likely to lower TSH results. The converse is also true—the dopamine antagonists (metoclopramide) may increase TSH concentrations.

Radioactive Iodine Uptake Test

This test is used to detect the ability of the thyroid gland to trap and concentrate iodine and, thereby, produce thyroid hormone. In other words, this test assesses the intrinsic function of the thyroid gland. This test is not specific, and its reference range must be adjusted to the local population. Therefore, its use is declining. In patients with a normal thyroid gland, 12–20% of the radioactive iodine is absorbed by the gland after six hours

MINICASE 3

A Case of Possible Hypothyroidism

Amy T., a 28-year-old female, visits her physician with complaints of weakness, fatigue, weight gain, hoarseness, cold intolerance, and unusually heavy periods worsening over the past two to three months. Her HR is 50 beats/min, and her BP is 110/70 mm Hg. Her physical exam is normal, except for a mildly enlarged thyroid gland, pallor, and diminished tendon reflexes. She denies taking any medications or changing her diet. Amy T.'s chemistry results are as follows:

- Sodium, 130 mEq/L (136–142 mEq/L)
- Potassium, 3.8 mEq/L (3.8–5 mEq/L)
- Carbon dioxide, 28 mEq/L (21–28 mEq/L)
- Calcium, 9.5 mg/dL (9.2–11 mg/dL)
- Magnesium, 2 mEq/L (1.3–2.1 mEq/L)
- Glucose, 80 mg/dL (70–110 mg/dL)
- BUN, 20 mg/dL (8–23 mg/dL)
- SCr, 1.1 mg/dL (0.6–1.2 mg/dL)
- Cholesterol, 235 mg/dL (<200 mg/dL)

The cholesterol concentration is elevated since a screening six months ago. A test for mononucleosis is negative. Hematocrit is low at 36% (36–45%)—close to her usual. Her total serum T_4 is 8 mcg/dL (5.5–12.5 mcg/dL), her T_3 resin uptake is 15% (25–38%), and her free T_4 index is 1.2 (1–4).

QUESTION: How should these results be interpreted? Are confirmatory tests needed?

DISCUSSION: Clinically, all of the history and physical findings point to hypothyroidism. The pallor and weakness are also consistent with anemia, but an Hct of 35% is unlikely to cause such significant symptoms. Her cholesterol recently became elevated, consistent with primary hypothyroidism.[36] Classically, both the total serum T_4 and T_3 resin uptake should be low in hypothyroid patients. In this patient, only the T_3 resin uptake is low, and the free T_4 index is borderline normal, making laboratory diagnosis unclear. Confirmatory tests should prove useful.

A few days later, she revisits her physician for additional tests. When questioned, she admits that she has been taking oral contraceptives and would like to continue. The following day, her TSH is 25 milliunits/L (0.3–5 milliunits/L).

QUESTION: Does this information help to elucidate the diagnosis?

DISCUSSION: An elevated TSH confirms primary hypothyroidism. The reason for equivocal total serum T_4 and T_3 resin uptake is now apparent—the estrogens in the birth control pills. Estrogens elevate total serum T_4 and thyroid-binding protein and lower T_3 resin uptake. If she had not been taking estrogens, her total serum T_4 probably would have been below normal, and her T_3 resin uptake probably would have been higher (but still below normal). The diagnosis would have been clear earlier. If oral contraceptive use had been identified at the first visit, a TSH concentration should have been performed then.

She is started on levothyroxine 0.125 mg/day, and her TSH is 6 milliunits/L three weeks later. Clinically, she improves but is not fully back to normal. Six weeks after starting therapy, she complains of jitteriness, palpitations, and increased sweating. Her TSH is <0.3 milliunit/L. Her physician lowers the dose of levothyroxine to 0.1 mg/day, and she becomes asymptomatic after about two weeks. Eight weeks later, her TSH is 1.5 milliunits/L, and she remains asymptomatic. Her cholesterol is 100 mg/dL, sodium is 138 mEq/L, and Hct is 40%.

QUESTION: Which test(s) should be used to determine proper dosing of levothyroxine? How long after a dosage change should clinicians wait before repeating the test(s)?

DISCUSSION: Although total serum T_4, T_3 resin uptake, and free T_4 index can be used to monitor and adjust doses of thyroid supplements in patients with a hypothyroid disorder, the highly sensitive TSH is most reliable. Chemically, the goal is to achieve a TSH in the normal range, as was ultimately achieved in this patient (TSH of 1.5 milliunits/L). Because of her continued use of birth control pills, TSH is the best test for her. The newer TSH assays make it possible to determine whether TSH secretion is excessively suppressed by thyroid replacement (<0.5 milliunit/L).

With the increased availability of this sensitive test, TSH is becoming the standard for adjusting thyroid replacement therapy in most patients. The 0.2-mg levothyroxine dose is excessive, as evidenced by her "hyperthyroid" symptoms and the fully suppressed TSH. Eight weeks later, after T_4 steady-state has been reached on the 0.1-mg/day dose and after the hypothalamic-pituitary-thyroid axis reached homeostasis, TSH is within the desired range. Her cholesterol, sodium, and Hct is also normalized when she became euthyroid.

and 5–25% is absorbed after 24 hours. The radioactive iodine uptake test is an indirect measure of thyroid gland activity and should not be used as a basic screening test of thyroid function. This test is most useful in distinguishing hyperthyroidism caused by subacute thyroiditis with absent or reduced uptake of iodine.[102,121,122]

A high radioactive iodine uptake is noted with the following[87,110,111]:

- Thyrotoxicosis
- Iodine deficiency
- Post-thyroiditis
- Withdrawal rebound after thyroid hormone or antithyroid drug therapy

A low test result occurs in the following[103,122,123]:

- Acute thyroiditis
- Euthyroid patients who ingest iodine-containing products
- Patients on exogenous thyroid hormone therapy
- Patients who are taking antithyroid drugs such as propylthiouracil
- Hypothyroidism

MINICASE 4

A Patient with Hyperthyroidism

Marie M., a 35-year-old housewife complains of nervousness, weakness, and palpitations with exertion for the past six months. Recently, she noticed excessive sweating and wanted to sleep with fewer blankets than her husband. She has lost 15 pounds over the last six months in spite of eating twice as much as she did one year ago. Her menstrual periods have been regular with less bleeding. Her HR is 96 beats/min and BP is 150/90 mm Hg. She appears anxious. She has smooth, warm, moist skin; a fine tremor; and she cannot rise from a deep knee bend without aid. Upon physical exam, her thyroid contains three nodules—two on the right and one on the left with a total gland size of 60 g (three times normal size). All nodules are firm consistency, and there is no lymphadenopathy.

- Sodium, 145 mEq/L (136–142 mEq/L)
- Potassium, 4 mEq/L (3.8–5 mEq/L)
- Chloride, 101 mEq/L (95–103 mEq/L)
- Carbon dioxide, 26 mEq/L (21–28 mEq/L)
- BUN, 10 mg/dL (8–23 mg/dL)
- SCr, 0.8 mg/dL (0.6–1.2 mg/dL)
- Hgb, 12 g/dL (12–16 g/dL)
- Hct, 36% (36–45%)
- RBC count, 3.5 M/mm^3 (4–5.2 M/mm^3)
- Antithyroid antibodies, 1:200
- MCV, 104 mm^3 (80–100)
- WBC count, 16 × 10^3 cells/mm^3 (4.8–10.8 × 10^3 cells/mm^3)
- Calcium, 9 mg/dL (9.2–11 mg/dL)
- Glucose, 96 mg/dL (70–110 mg/dL)
- Free T_4, 15.6 ng/dL (0.9–2.3 ng/dL)
- T_3, 250 ng/dL (80–200 ng/dL)
- TSH, 0.3 microunits/mL (0.5–5 microunits/mL)

QUESTION: How should these results be interpreted? Are confirmatory tests needed?

DISCUSSION: This patient presents with many of the clinical features of hyperthyroidism. These include rapid heart rate, weight loss, and heat intolerance. Her thyroid gland is visibly enlarged (goiter). She also has elevated blood pressure and complains of nervousness, sweating, and hand tremors. The diagnosis of hyperthyroidism can be confirmed by her laboratory results of a high T_4 and a suppressed TSH value. She has a toxic multinodular goiter that should be treated with radioactive iodine or surgery with antithyroid drug and iodine pretreatment.

The radioactive iodine uptake test is affected by the body's store of iodine. Therefore, the patient should be carefully questioned about the use of iodine-containing products prior to the test. This test is contraindicated during pregnancy.

Antithyroid Antibodies

Normal range: varies with antibody

Antibodies that "attack" various thyroid tissue components can be detected in the serum of patients with autoimmune disorders such as Hashimoto thyroiditis and Graves disease. Thyroid microsomal antibody is found in 95% of patients with Hashimoto thyroiditis, 55% of patients with Graves disease, and 10% of adults without thyroid disease. In patients who have nodular and hard goiters, high-antibody titers strongly suggest Hashimoto thyroiditis as opposed to cancer. In Graves disease, hyperthyroidism is caused by antibodies, which activate TSH receptors. In chronic autoimmune thyroiditis, hypothyroidism may be caused by antibodies competitively binding to TSH receptors, thereby blocking TSH from eliciting a response.[123]

Results are reported as titers. Titers in excess of 1:100 are significant and usually can be detected even during remission. Antibodies (>1:10) to thyroglobulin are present in 60–70% of adults with active Hashimoto thyroiditis, but typically are not detected during remission. Titers above 1:1000 are found only in Hashimoto thyroiditis or Graves disease (25–10%, respectively). Lower titers may be seen in 4% of the normal population, although the frequency increases with the age in females. The thyroid microsomal antibody and thyroglobulin antibody serological tests may be elevated or positive in patients with nonthyroidal autoimmune disease.

Anti-TSH receptor antibodies are present in virtually all patients with Graves disease, but the test is usually not necessary for diagnosis. These antibodies mostly stimulate TSH receptors but also may compete with TSH and inhibit TSH stimulation of the thyroid gland. High titers allow a confirmation of Graves disease in asymptomatic patients, such as those whose only manifestation is exophthalmos (**Minicase 5**).

Laboratory Diagnosis of Hypothalamic-Pituitary-Thyroid Axis Dysfunction

The laboratory diagnosis of primary hypothyroidism can be made with a low free T_4 index and an elevated TSH concentration. The presence of a low free T_4 index and a normal or low serum TSH concentration indicates secondary or tertiary hypothyroidism or nonthyroid illness. In such patients, the T_3 resin uptake may differentiate between hypothyroidism and a low T_4 state due to nonthyroid illness. An elevated reverse T_3 concentration also suggests nonthyroid illness. T_3 is of limited usefulness in diagnosing hypothyroidism because it may be normal in up to one third of hypothyroid patients.[102,104,121,122] With the availability of ultrasensitive TSH assays, many clinicians begin their evaluations with this test.

Newer generation TSH assay can also be used to diagnose hyperthyroidism. The total serum T_4 and free T_4 or free T_4 index are commonly used and are increased in almost all hyperthyroid patients. Usually, both T_3 and T_4 are elevated.

MINICASE 5

A Patient with Abnormal TSH Test Results

Rita T., a 27-year-old female being assessed for infertility, is found to have a TSH concentration of 8.2 milliunits/L (0.5–5.0 milliunits/L) as measured by a second-generation assay. Her physical exam reveals no abnormalities, and she is clinically euthyroid. Her total serum T_4 is 10 mcg/dL (5.5–12.5 mcg/dL). She is treated with 0.125 mg/day of levothyroxine for one month, and a repeat TSH is 6.8 milliunits/L. Her dose is increased to 0.2 mg/day.

After two months, Rita T.'s TSH is 8.8 milliunits/L, while her total serum T_4 is 15 mcg/dL, her total serum T_3 is 200 ng/dL (80–200 ng/dL), her TBG is 32 nmol/L (10–26 nmol/L), and her free T_3 is 4.7 pmol/L (3.2–5.1 pmol/L). She is slightly hyperthyroid. Levothyroxine therapy is stopped and a TRH challenge (200 mcg IV) that same day evokes only minimal increases in TSH concentrations. An MRI of the hypothalamic-pituitary area is normal.

QUESTION: What could have caused this patient's initial elevated TSH?

DISCUSSION: Although TSH is classically elevated in patients with primary hypothyroidism, she does not have any clinical signs or symptoms of this disorder. Inappropriate elevation of TSH can be caused by a TSH-secreting pituitary tumor, thyroid hormone resistance, or assay interference. Tumor is ruled out by the normal MRI. Hormone resistance is not consistent with her picture. If she has pituitary-confined resistance, persistent secretion of TSH and thyroid hormones could lead to clinical hyperthyroidism. If resistance has been general, she might be euthyroid, but T_4 and T_3 concentrations would be elevated along with TSH.[38]

Finally, transiently elevated TSH may be found in patients recovering from major physiological stress (e.g., intensive care illnesses and trauma), which is not the case here. Thyroid status should be evaluated after major medical problems have stabilized. The TRH challenge shows essentially no response, a finding that reflects the iatrogenic hyperthyroidism at the time. Therefore, by exclusion, her elevated TSH concentration is most likely an artifact, probably due to interfering antibodies. This condition is rare but has been described.

However, a few (<5%) hyperthyroid patients exhibit normal T_4 with elevated T_3 (T_3 toxicosis). Second-line tests such as antithyroid antibody serologies are necessary to diagnose autoimmune thyroid disorders.

ADRENAL DISORDERS

The adrenal glands are located extraperitoneally at the upper poles of each kidney. The adrenal medulla, which makes up 10% of the adrenal gland, secretes catecholamines (e.g., epinephrine and norepinephrine). The adrenal cortex, which comprises 90% of the adrenal gland, is divided into three areas:

1. The outer layer of the adrenal gland, known as the *zona glomerulosa*, makes up 15% of the adrenal gland and is responsible for production of aldosterone, a mineralocorticoid that regulates electrolyte and volume homeostasis.
2. The *zona fasciculata,* located in the center of the adrenal gland, occupies 60% of the gland and is responsible for glucocorticoid production. Cortisol, a principal end product of glucocorticoid production, regulates fat, carbohydrate, and protein metabolism. Glucocorticoids maintain the body's homeostasis by regulating bodily functions involved in stress as well as normal activities.
3. The *zona reticularis* makes up 25% of the adrenal gland and is responsible for adrenal androgens such as testosterone and estrogens such as estradiol. These hormones influence the development of the reproductive system.[101]

Cushing Syndrome

Cushing syndrome, first described 70 years ago, is the result of excessive concentrations of cortisol. In most cases, hypercortisolism is the result of overproduction of cortisol by the adrenal glands due to an adrenocorticotropic hormone (ACTH) secreting pituitary tumor. Adrenal tumors and long-term use of glucocorticoids can also result in hypercortisolism.

Patients with hypercortisolism generally present with facial plethora (moon face) as a result of atrophy of the skin and underlying tissue. A common sign of hypercortisolism is fat accumulation in the dorsocervical area often referred to as "buffalo hump." Other cardinal signs and symptoms include hypertension, osteopenia, glucose intolerance, myopathy, bruising, and depression. Hyperpigmentation is present in patients with ACTH-secreting pituitary tumors. Hair loss, acne, and oligomenorrhea are also the result of superfluous cortical secretion.[127]

Diagnostic tests. The following tests are employed to identify patients with Cushing syndrome: 24-hour urine-free cortisol (UFC), midnight plasma cortisol, and the low-dose dexamethasone suppression test (DST) using 1 mg for the overnight test or 0.5 mg every six hours for the two-day study. The most frequently used test to identify patients with hypercortisolism is the 24-hour UFC test, which measures free cortisol levels and creatinine in a urine sample that is collected over a 24-hour period. Laboratory results for these tests in adults with Cushing syndrome include the following:

Of the suppression tests, the overnight DST is the least laborious test to perform. The patient is given 1 mg of dexamethasone at 11:00 p.m. A plasma cortisol levels is obtained at 8:00 a.m. the next morning. Patients with Cushing syndrome will have high cortisol concentrations (>5 mcg/dL or >138 nmol/L) due to an inability to suppress the negative-feedback mechanism of the hypothalamic-pituitary-adrenal (HPA) axis.[127-129]

Once hypercortisolism is confirmed, one of the following tests should be performed to identify the source of hypersecretion, which could include the pituitary gland, adrenal gland, or production from an ectopic site. Such tests include high-dose DST; plasma ACTH via immunoradiometric assay (IRMA) or RIA; adrenal vein catheterization; metyrapone stimulation test; adrenal, chest, or abdominal computed tomography (CT); corticotropin-releasing hormone (CRH) stimulation test; inferior petrosal sinus sampling; and pituitary magnetic resonance imaging (MRI). Other possible tests and procedures include insulin-induced hypoglycemia, somatostatin receptor scintigraphy; desmopressin stimulation test; naloxone CRH stimulation test; loperamide test; hexarelin stimulation test; and radionuclide imaging. Additional tests should be performed to confirm the diagnosis because other factors (e.g., starvation, topical steroid application, and acute stress) influence the results of the above mentioned tests.[127]

Plasma ACTH concentrations can be measured by RIA procedures. Interpretation of the results is as follows:

- ACTH levels <5 pg/mL indicate an ACTH-independent adrenal source, such as an adrenal tumor or long-term use of steroids.
- ACTH levels between 5–15 pg/mL should be followed by a CRH test.
- ACTH levels >15 pg/mL indicate an ACTH-dependent syndrome.

The CRH test can be employed to determine if the source of hypercortisolism is pituitary or ectopic (extra pituitary). Baseline ACTH and CRH levels are obtained. Then, ACTH and cortisol levels are measured 15–30 and 45–60 minutes after the administration of a 100-mcg IV dose of CRH. A 50% increase from baseline in ACTH levels indicates an ACTH-dependent syndrome.[127]

• 24-hour UFC	at least three times above normal
• Midnight plasma cortisol	5 mcg/dL (130 nmol/L) or more
• Low-dose DST	plasma cortisol exceeds 2 mcg/dL (50 nmol/L) when drawn between 8:00 and 9:00

Pharmacologic treatment. Treatment of Cushing syndrome is dependent on the cause. If due to a pituitary adenoma, transsphenoidal surgery may be indicated. If due to adrenal hyperplasia, an adrenalectomy may be needed. If due to an adrenal corticol carcinoma, inhibition of steroid synthesis with mitotane is frequently used. Mitotane inhibits steroid biosynthesis as well as inhibits peripheral steroid metabolism and cortisol release.[128] Alternative agents, metyrapone, aminoglutethimide, and ketoconazole are adrenal enzyme inhibitors that inhibit enzymes necessary for the conversion of cholesterol into steroid hormones, which is a necessary step for the production of cortisol. Cyproheptadine is a neuromodulatory agent that has been used to decrease ACTH secretion. Because of a low response rate of <30%, cyproheptadine is reserved for patients who fail conventional therapy.[128,129]

Adrenal Insufficiency (Addison Disease)

Adrenal insufficiency (Addison disease or *primary adrenal insufficiency)* is the result of an autoimmune destruction of all regions of the adrenal cortex. Tuberculosis, fungal infections, acquired immunodeficiency syndrome, metastatic cancer, and lymphomas can also precipitate adrenal insufficiency. Adrenal insufficiency results in deficiencies in cortisol, aldosterone, and androgens. Patients usually present with weakness, weight loss, increased pigmentation, hypotension, GI symptoms, postural dizziness, and vertigo.

Secondary adrenal insufficiency can result from the use of high doses of exogenous steroids, which suppress the hypothalamic-pituitary axis resulting in a decrease in the release of ACTH. Patients with secondary adrenal insufficiency maintain normal aldosterone levels and do not exhibit signs of hyperpigmentation.[130,131]

Diagnostic tests. The cosyntropin stimulation test is used to diagnose patients with low cortisol levels. Patients are administered 250 mcg of synthetic ACTH or cosyntropin intravenously or intramuscularly. Serum cortisol levels are drawn at the time of injection and 30 minutes and one hour after the injection. Cortisol levels >18–20 mcg/dL (497–552 nmol/L) indicate an adequate response from the adrenal gland, thus ruling out adrenal insufficiency. The cosyntropin stimulation test may be normal in patients with secondary adrenal insufficiency or mild primary adrenal insufficiency due to the high dose of corticotropin given. In light of this, many endocrinologists recommend that higher cutoff values (≥22–25 mcg/dL or 607–690 nmol/L) be used. To distinguish primary from secondary adrenal insufficiency, ACTH, renin, and aldosterone levels are measured.

Pharmacologic treatment. Corticosteroids are used to treat adrenal insufficiency. The agents of choice include prednisone 5 mg/day, hydrocortisone 20 mg/day, or cortisone 25 mg/day given in the morning and evening. Additional mineralocorticoid (e.g., fludrocortisone acetate 0.05–2.0 mg daily) should be given to patients with primary adrenal insufficiency because there is a concomitant decrease in aldosterone production. The endpoint of therapy is the reversal of signs and symptoms of adrenal insufficiency, particularly excess pigmentation.[132]

DIABETES INSIPIDUS

Diabetes insipidus is a syndrome in which the body's inability to conserve water manifests as excretion of very large volumes of dilute urine. This section explores related pathophysiology, types of diabetes insipidus, and interpretation of test results to evaluate this disorder.

Physiology

Normally, serum osmolality is maintained around 285 mOsm/kg and is determined by the amounts of sodium, chloride, bicarbonate, glucose, and urea in the serum. The excretion of these solutes along with water is a primary factor in determining urine volume and concentration. In turn, the amount of

TABLE 10-14. Differential Diagnosis of Diabetes Insipidus Based on Water Deprivation Test[133,134]

DIAGNOSIS	URINE SPECIFIC GRAVITY	AVERAGE URINE OSMOLALITY (mOsm/kg)	PLATEAU URINE OSMOLALITY (mOsm/kg)	AVERAGE SERUM OSMOLALITY (mOsm/kg)	CHANGE IN URINE OSMOLALITY AFTER VASOPRESSIN
Normal individuals	>1.015	300–800	<1600	280–295	Little change
Central diabetes insipidus	<1.010	<300	<300	Normal or increased	Increases
Nephrogenic diabetes insipidus	<1.010	<300	<300	Normal or increased	Little change

water excreted by the kidneys is determined by renal function and ADH (vasopressin). ADH reduces renal elimination of water and produces concentrated urine.

ADH is synthesized in the hypothalamus and stored in the posterior pituitary gland. This hormone is released into the circulation following physiological stimulation, such as an increase in serum osmolality or blood volume detected by the osmoregulatory centers in the hypothalamus.[133,134] Congestive heart failure lowers the osmotic threshold for ADH release, while nausea—but not vomiting—strongly stimulates ADH. In general, α-adrenergic agonists stimulate ADH release, while β-adrenergic agonists inhibit release, and acts on the distal renal tubule and the collecting duct to cause water reabsorption. Chlorpropamide potentiates the effect of ADH on renal concentrating ability. When ADH is lacking or the renal tubules do not respond to the hormone, polyuria ensues. If the polyuria is severe enough, a diagnosis of diabetes insipidus is considered.[134,135]

Clinical Diagnosis

Diabetes insipidus should be differentiated from other causes of polyuria such as osmotic diuresis (e.g., hyperglycemia, mannitol, and contrast media), renal tubular acidosis, diuretic therapy, and psychogenic polydipsia. Patients usually excrete 16–24 L of dilute urine in 24 hours. The urine specific gravity is <1.005 and urine osmolality <300 mOsm/kg.[135,136] As long as the thirst mechanism is intact and the patient can drink, no electrolyte problems result. However, if the patient is unable to replace fluids lost through excessive urine output, the patient can develop dehydration.

Although diabetes insipidus is usually caused by a defect in the pituitary secretion (neurogenic, also called *central*) or renal activity (nephrogenic) of ADH, it can also be caused by a defect in thirst (dipsogenic) or psychological function (psychogenic), with resultant excessive intake of water. Although diabetes insipidus typically does not lead to significant morbidity, the underlying cause should be sought to ensure proper diagnosis and therapy. The specific type of diabetes insipidus often can be identified by the clinical setting. If the diagnosis is equivocal, a therapeutic trial with an antidiuretic drug or measurement of plasma ADH is necessary (**Table 10-14**).[136]

Central Diabetes Insipidus

Central diabetes insipidus (ADH deficiency) may be the result of any disruption in the pituitary–hypothalamic regulation of ADH. Patients often present with a sudden onset of polyuria (in the absence of hyperglycemia) and preference for iced drinks. Tumors or metastases in or around the pituitary or hypothalamus, head trauma, neurosurgery, genetic abnormalities, Guillain-Barré syndrome, meningitis, encephalitis, toxoplasmosis, cytomegalovirus, tuberculosis, and aneurysms are some of the known causes. In addition, phenytoin and alcohol inhibit ADH release from the pituitary. In response to deficient secretion of ADH and subsequent hyperosmolality of the plasma, thirst is stimulated. Thirst induces water intake, which leads to polyuria in the absence of effective ADH.[134]

Nephrogenic Diabetes Insipidus

In *nephrogenic diabetes insipidus* (ADH resistance), the secretion of ADH is normal, but the renal tubules do not respond to ADH. Causes of nephrogenic diabetes insipidus include chronic renal failure, pyelonephritis, hypokalemia, hypercalciuria, malnutrition, genetic defects, and sickle cell disease. Additionally, lithium toxicity, colchicine, glyburide, demeclocycline, cidofovir, and methoxyflurane occasionally cause this disorder.

Lithium leads to polyuria in about 20% of patients. Typically, polyuria occurs after two to three months of therapy. Lithium appears to exert its nephrotoxicity by entering collecting duct cells through sodium channels. Lithium impairs ADH's ability to produce cyclic adenosine monophosphate, resulting in resistance to the renal effects of ADH on the collecting duct and water loss. Sodium reabsorption in the cortical diluting and distal tubules results in increased urine output. Amiloride, a potassium-sparing diuretic, is useful at doses of 5 mg/day in lithium-induced diabetes insipidus because it closes the sodium channels in the collecting duct cells and decreases lithium accumulation. Chlorpropamide potentiates ADH's effect on the collecting tubules. Chlorpropamide is usually given in doses of 125–500 mg daily; patients should be monitored for hypoglycemia. Thiazides can be used to block sodium reabsorption in the cortical diluting tubule and the distal tubule, thereby, decreasing urine output. Hydrochlorothiazide 50–100 mg daily or an equivalent dose of another thiazide diuretic can be used. Patients who are treated with thiazide diuretics must be monitored because these agents can cause hypokalemia and hypomagnesemia.[134]

Diabetes Insipidus of Pregnancy

A transient diabetes insipidus, originally thought to be a form of nephrogenic diabetes insipidus, may develop during late pregnancy from excessive vasopressinase (ADHase) activity. This kind of diabetes insipidus is associated with preeclampsia with liver involvement. Fortunately, vasopressinase does not metabolize DDAVP (desmopressin acetate), which is, therefore, the treatment of choice.[135]

Laboratory diagnosis. Some clinicians avoid dehydration testing and rely on measuring plasma ADH concentrations to distinguish central from nephrogenic diabetes insipidus. In otherwise healthy adults, the average basal plasma ADH concentration is 1.3–4 pg/mL (1.2–3.7 pmol/L). Based on medical history, symptoms, and signs, an elevated basal plasma ADH level almost always indicates nephrogenic diabetes insipidus. If the basal plasma ADH concentration is low (<1 pg/mL) or immeasurable, the result is inconclusive and a dehydration test should be done.

The theory behind the water deprivation test is that, in normal individuals, dehydration stimulates ADH release and the urine becomes concentrated. An injection of vasopressin at this point does not further concentrate the urine. In contrast, the urine of patients with central diabetes insipidus will not be maximally concentrated after fluid deprivation but will be after vasopressin injection.

To perform the test, patients are deprived of fluid intake (up to 18 hours) until the urine osmolality of three consecutive samples varies by no more than 30 mOsm/kg. Urine osmolality and specific gravity are measured hourly. At this time, 5 units of aqueous vasopressin are administered subcutaneously, and urine osmolality is measured one hour later. Plasma osmolality is measured before the test, when urine osmolality has stabilized, and after vasopressin has been administered.

In healthy individuals, fluid deprivation for 8-hour to 12-hour results in normal serum osmolality and a urine osmolality of about 800 mOsm/kg. The urine osmolality plateaus after 16–18 hours. Patients with central diabetes insipidus have an immediate rise in urine osmolality to approximately 600 mOsm/kg, with a corresponding decrease in urine output with vasopressin injection. Patients with nephrogenic diabetes insipidus are unable to increase urine osmolality above 300 mOsm/kg as vasopressin injection has little effect.

In addition to being inconvenient and expensive, dehydration procedures are reliable only if the diabetes insipidus is severe enough that—even with induced dehydration—the urine still cannot be concentrated.

Accurate interpretation requires consideration of potential confounding factors. If the laboratory cannot ensure accurate and precise plasma (not serum) osmolality measurements, plasma sodium should be used. Patients should be observed for nonosmotic stimuli, such as vasovagal reactions, that may affect ADH release. Lastly, if the patient has previously received ADH therapy, ADH antibodies may cause false-positive results suggestive of nephrogenic diabetes insipidus.[137]

SUMMARY

Endocrine disorders often result from a deficiency or excess of a hormone. Laboratory tests that measure the actual hormone, precursors, or metabolites can help to elucidate whether and why a hormonal or metabolic imbalance exists. Tests used to assess thyroid, adrenal, glucose, and water homeostasis or receptors have been discussed.

The FPG and the two-hour PPG concentrations are the most commonly performed tests for evaluation of glucose homeostasis. If elevated (>126 mg/dL) blood glucose persists, DM is likely. However, other causes of hyperglycemia (e.g., drugs) should be considered. Glycated hemoglobin assesses average glucose control over the previous two to three months, while fructosamine assesses average control over the previous two to three weeks.

DKA and hyperosmolar nonketotic hyperglycemia are the most severe disorders along the continuum of glucose intolerance. Extreme hyperglycemia (600–2000 mg/dL) with insignificant ketonemia/acidosis is consistent with hyperosmolar nonketotic hyperglycemia, while less severe (350–650 mg/dL) hyperglycemia with ketonemia and acidosis is characteristic of DKA. Conversely, hypoglycemia (glucose <50 mg/dL) most often is seen in patients with type 1 DM who have injected excessive insulin relative to their caloric intake.

Thyroid tests can be divided into those that (1) measure the concentration of products secreted by the thyroid gland (T_3 and T_4); (2) evaluate the integrity of the hypothalamic-pituitary-thyroid axis (TSH and TRH); (3) assess intrinsic thyroid gland function (radioactive iodine uptake test); and (4) detect antibodies to thyroid tissue (thyroid microsomal antibody). Although TSH concentrations are usually undetectable or <0.3 milliunit/L in patients with hypothyroidism, T_4 concentrations are usually high in patients with overt hyperthyroidism. The TSH concentrations are low or undetectable in patients with hypothyroidism from hypothalamic or pituitary insufficiency and in patients with nonthyroidal illness. In contrast, TSH concentrations are high and T_4 concentrations are low in patients with primary hypothyroidism.

Glucocorticoids maintain the body's homeostasis by regulating bodily functions involved in stress and normal activities. Cortisol, androgens, aldosterone, and estrogens are all produced in the adrenal glands. Cushing syndrome is the result of excessive cortisol in the body. Addison disease occurs when there is a deficiency in cortisol production.

Diabetes insipidus is a syndrome in which the body's inability to conserve water manifests as excretion of very large volumes of dilute urine. It most often is caused by a defect in the secretion (neurogenic, also called *central*) or renal activity (nephrogenic) of ADH. Urine and plasma osmolality are key tests. With the advent of high-performance assays, the use of plasma vasopressin concentrations to distinguish central from nephrogenic types may obviate the need for provocative iatrogenic dehydration testing procedures.

LEARNING POINTS

1. ***Which patients with diabetes benefit from SMBG and how can different test results (premeal, postmeal, and fasting) be used in diabetes management?***

   ANSWER: The ADA recommends SMBG for all people with diabetes who use insulin.[18] Self-monitoring provides information patients can use to adjust insulin doses, physical activity, and carbohydrate intake in response to high or low glucose levels. The goal of SMBG is to prevent hypoglycemia, while maintaining blood glucose levels as close to normal as possible. Most people with type 1 DM must use SMBG to achieve this goal. Although patients with type 2 DM receiving insulin therapy benefit from SMBG, the benefit of SMBG for individuals with type 2 DM who do not use insulin is not firmly established.[137,138] The ADA states that SMBG may be desirable in patients treated with sulfonylureas or other drugs that increase insulin secretion.[137] The frequency and timing of SMBG vary based on several factors, including an individual's glycemic goals, the current level of glucose control, and the treatment regimen. The ADA recommends SMBG three or more times per day for most individuals who have type 1 DM and pregnant women who use insulin. More frequent testing (four to six times per day) may be needed to monitor pump therapy.[18] In patients with type 1 DM, SMBG is most commonly recommended four times a day, before meals and at bedtime. A periodic 2:00 a.m. test is recommended to monitor for nighttime hypoglycemia. These measurements are used to adjust insulin doses and attain the fasting glucose goal. However, there is evidence that blood glucose measurements taken after lunch, after dinner, and at bedtime have the highest correlation to A1c values.[138] When premeal or fasting goals are reached but A1c values are not optimal, SMBG two hours after meals can provide guidance for further adjustment of insulin regimens. Postmeal measurements are also used to evaluate the effects of rapid-acting insulins (e.g., lispro, aspart), which are injected just before meals. Type 2 DM patients who use multiple daily injections of insulin should generally test as often as type 1 patients (at least three times per day). Patients on once-daily insulin and oral medications may also benefit from testing before meals and at bedtime when therapy is initiated or if control is poor.[140,141]

2. ***What factors should a pharmacist consider when helping a patient select an SMBG meter?***

   ANSWER: Meters offer a variety of features that should be considered in the selection process. The key features are meter size; the amount of blood required for each test; ease of use; speed of testing; cleaning and calibration requirements; alternate site testing capability; meter and test strip cost; language choice; and the capability to store readings, average readings over time, and download data. Patient factors to consider include lifestyle (where they will be testing; importance of portability and speed), preferences (importance of small sample size or alternate site capability), dexterity (can they operate the meter), visual acuity, and insurance coverage.[142]

3. ***How often should people with diabetes have their A1c level tested?***

   ANSWER: According to the ADA, people who are meeting their treatment goals and who have stable blood glucose control should get A1c testing at least twice a year. People whose treatment has been changed recently or who are not achieving their blood glucose targets should be tested four times a year.[28,29]

4. ***What factors may affect the accuracy of a A1c result?***

   ANSWER: False elevations in A1c may be noted with uremia, chronic alcohol intake, and hypertriglyceridemia. Patients who have diseases with chronic or episodic hemolysis (e.g., sickle cell disease and thalassemia) generally have spuriously low A1c concentrations caused by the predominance of young RBCs (which carry less A1c) in the circulation. In splenectomized patients and those with polycythemia, A1c is increased. If these disorders are stable, the test still can be used, but values must be compared with the patient's previous results rather than published normal values. Both falsely elevated and falsely lowered measurements of A1c may also occur during pregnancy. Therefore, it should not be used to screen for gestational DM.[32,34]

5. ***Which laboratory tests are recommended in the initial evaluation of thyroid disorders?***

   ANSWER: The principal laboratory tests recommended in the initial evaluation of a suspected thyroid disorder are the sensitive TSH and the free T_4 levels. Free T_4 is the most accurate reflection of thyrometabolic status. The free T_4 is the most reliable diagnostic test for the evaluation of hypothyroidism and hyperthyroidism when thyroid hormone binding abnormalities exist. If a direct measure of the free T_4 level is not available, the estimated free T_4 index can provide comparable information. Total serum T_4 is still the standard initial screening test to assess thyroid function because of its wide availability and quick turnaround time. In most patients, the total serum T_4 level is a sensitive test to evaluate the function of the thyroid gland. This test measures both bound and free T_4 and is, therefore, less reliable than the free T_4 or free T_4 index when alterations in TBG or nonthyroidal illnesses exist. The serum TSH is the most sensitive test to evaluate thyroid function. TSH secreted by the pituitary is elevated in early or subclinical hypothyroidism (when thyroid hormone levels appear normal) or when thyroid hormone replacement therapy is inadequate.[143]

APPENDIX A. Antidiabetic Agents[2,66-69]

CLASS	AGENT	MECHANISM	SIDE EFFECTS	DOSAGE RANGE AND ROUTE OF ADMINISTRATION	LABORATORY TESTS FOR MONITORING
Sulfonylureas		Increases insulin secretion by binding to sulfonylurea receptor on pancreatic β-cell and blocking ATP-K channels	Hypoglycemia, weight gain, water retention, hematologic reactions, skin reactions (particularly rashes), purpura, nausea, vomiting and cholestasis, constipation, headache, photosensitivity		Directly affects FPG and PPG; A1c should be monitored to assess overall glycemic control
1st generation	Chlorpropamide (Diabinese)		Disulfiram-like reaction when combined with alcohol; excessive hypoglycemia in patients with renal insufficiency so avoid use	100–500 mg PO daily; max: 750 mg/day	
	Tolazamide (Tolinase)			100–1000 mg PO in one or two divided doses daily	
	Tolbutamide (Orinase)			500–2500 mg PO in one to three divided doses daily	
2nd generation	Glipizide (Glucotrol)		Increased risk of rash when compared to other sulfonylureas	5–10 mg PO daily; titrate to max dose of 40 mg daily; usually not above 20 mg/day; give 30 min before meals	
	Glyburide (Diabeta, Glynase, Micronase)		Dizziness, blurred vision; avoid in patients with renal insufficiency (do not use if CrCl <50 mL/min)	5 mg PO daily; titrate to max dose of 20 mg daily; usually not above 10 mg/day	
	Glimepiride (Amaryl)		Mild incidence of hypoglycemia, fullness, heartburn, blood dyscrasias	1–2 mg PO daily; titrate to maintenance; usually not above 8 mg/day	
D-phenylalanine derivative	Nateglinide (Starlix)	Increases insulin secretion by blocking ATP-K channels on the pancreatic β cells	Mild hypoglycemia (primarily postprandial), nausea, diarrhea, weight gain	60–360 mg PO daily; give before meals	Directly affects PPG; FPG and A1c should be monitored to assess overall glycemic control
Meglitinide	Repaglinide (Prandin)	Increases insulin secretion by blocking ATP-K channels on the pancreatic β cells	Mild hypoglycemia (primarily postprandial), nausea, diarrhea, upper respiratory infection, headache, rhinitis, bronchitis, back pain, tooth disorder, chest pain, hyperglycemia, heartburn, epigastric fullness, weight gain	0.5–2 mg (max 4 mg) should be taken 15 min prior to meals, but may vary from immediately preceding a meal to as long 30 min prior; dosed up to four times daily in response to meal pattern; max: 16 mg/day	Directly affects PPG; FPG and A1c should be monitored to assess overall glycemic control
Biguanide	Metformin (Glucophage)	Inhibits gluconeogenesis, increases insulin sensitivity by increasing tyrosine kinase activity, increases glycogen synthesis	GI side effects including abdominal discomfort and diarrhea are most common; interference with vitamin B_{12} absorption; rarely lactic acidosis (especially when hypoperfusion is present (e.g., renal impairment and heart failure); do not use if SCr ≥1.5 (males) or ≥1.4 (females) or eGFR <50 mL/min	500 mg PO once a day to start (decreases incidence of diarrhea), increase by 500 mg/day increments at weekly intervals as tolerated; given in divided dose q 8–12 hr; max dose: 2550 mg/day For extended-release formulations, start with 500 mg daily; increase by 500 mg/week at weekly intervals as tolerated to a maximum of 2000 mg/day (for Glumetza or Glucophage XR) or 2500 mg/day (for Fortamet)	FPG, A1c, SCr

APPENDIX A. Antidiabetic Agents[2,66-69], continued

CLASS	AGENT	MECHANISM	SIDE EFFECTS	DOSAGE RANGE AND ROUTE OF ADMINISTRATION	LABORATORY TESTS FOR MONITORING
α-glucosidase inhibitors		Inhibits α-glucosidase enzyme, which metabolizes complex carbohydrates and sucrose (cane sugar)	Bloating, abdominal discomfort/pain, diarrhea and flatulence in up to 30% of patients; abnormal liver function tests	25 mg PO daily with first bite of food; titrate slowly to three times a day; max dose 50 mg three times a day for patients <60 kg and 100 mg TID for patients >60 kg; do not take without a meal	Directly affects PPG; FPG and A1c should be monitored to assess overall glycemic control, liver function tests
	Acarbose (Precose)				AST and ALT q 3 mo for 1 yr
	Miglitol (Glyset)		Skin rash, no reports of hepatic toxicity to date		
Thiazolidinediones		Facilitates muscle cell response to insulin by acting on the PPAR, thereby allowing glucose to diffuse into the cell more effectively	Hepatotoxicity, edema; contraindicated in patients with congestive heart failure NYHA class III or IV, rash, weight gain, decrease in hemoglobin and hematocrit; possible increase in LDL cholesterol; potential increase in heart attacks and heart-related chest pain; do not use if ALT >2.5 × ULN		Directly affects PPG and FPG; A1c should be monitored to assess overall glycemic control liver function tests, hemoglobin, and hematocrit
	Rosiglitazone (Avandia)		Do not use in combination with insulin or nitrates (may increase risk of myocardial infarction)	4 mg PO daily; max dose of 8 mg daily; 4 mg BID more effective than 8 mg daily	
	Pioglitazone (Actos)		May increase risk of bladder cancer with long-term use (>2 yr use)	15–30 mg PO daily; max dose 45 mg; decrease insulin dose by 10–25% if patient develops hypoglycemia on the combination	
GLP-1 agonists		Analog of GLP-1, a naturally occurring peptide, which enhances insulin secretion in response to elevated plasma glucose levels	Hypoglycemia when combined with sulfonylureas; nausea is common; vomiting, diarrhea, dizziness, headache, jitteriness, weight loss GLP-1 agonists cause thyroid C-cell tumors at clinically relevant exposures in rats; it is unknown whether these agents cause thyroid C-cell tumors, including MTC, in humans; GLP-1 agonists are contraindicated in patients with a personal or family history of MTC or in patients with MEN2		
	Exenatide (Byetta)	Mimetic of GLP-1		Subcutaneous injection of 5–10 mcg BID 60 min before morning and evening meals; do not use if CrCl <30 mL/min	FPG, PPG, A1c
	Exenatide (Bydureon)	Mimetic of GLP-1		2-mg subcutaneous injection once a week; do not use if CrCL <30 mL/min	FPG, PPG, A1c

APPENDIX A. Antidiabetic Agents[2,66-69]

CLASS	AGENT	MECHANISM	SIDE EFFECTS	DOSAGE RANGE AND ROUTE OF ADMINISTRATION	LABORATORY TESTS FOR MONITORING
	Liraglutide (Victoza)	Analog of GLP-1		0.6-mg subcutaneous injection once a day; max daily dose of 1.8 mg daily	FPG, PPG, A1c
	Dulaglutide (Trulicity)			0.75–1.5 mg subcutaneous injection once a week	
	Albiglutide (Tanzeum)			30-mg subcutaneous injection once a week; dose may be increased to 50 mg	
DPP-4 inhibitor		Inhibits DPP-4; increases levels of GLP-1, suppresses glucagon secretion	Hypoglycemia when used in combination with sulfonylurea, nausea, diarrhea, headache, upper respiratory tract infection		Directly affects PPG; FPG and A1c should be monitored to assess overall glycemic control
	Sitagliptin (Januvia)			100 mg PO once daily (50 mg PO once daily if CrCl 30–50 mL/min, 25 mg PO once daily if CrCl <30 mL/min)	
	Saxagliptin (Onglyza)		Increase risk of heart failure, particularly in patients who already have heart or kidney disease	2.5–5 mg PO once daily (2.5 mg PO once daily if CrCl <50 mL/min)	
	Linagliptin (Tradjenta)			5 mg PO once daily; no dose modification for patients with renal Impairment	
	Alogliptin (Nesina)		Increase risk of heart failure, particularly in patients who already have heart or kidney disease	25 mg PO once daily (12.5 mg if CrCl 30–60 mL/min; 6.25 mg if CrCl 15–30 mg/min)	
Sodium–glucose cotransporter 2 inhibitors		SGLT2 inhibitors reduce hyperglycemia in an insulin-independent manner by inhibiting the reabsorption of glucose into the systemic circulation by the kidneys	Ketoacidosis, urinary tract infection, female genital mycotic infections, upper respiratory tract infection, increased urination, dyslipidemia, male genital mycotic infections, nausea, polydipsia		FPG, PPG, and A1c should be monitored to assess overall glycemic control; potassium levels in patients receiving ACE inhibitors, ARBs, or renal impairment
	Canagliflozin (Invokana)			100–300 mg PO once daily • eGFR 45 to <60 mL/min/1.73 m^2: do not exceed 100 mg/day • eGFR <45 mL/min/1.73 m^2: do not initiate canagliflozin • Discontinue if eGFR declines below 45 mL/min/1.73 m^2	
	Dapagliflozin (Farxiga)			5–10 mg PO once daily	
	Empagliflozin (Jardiance)			10–25 mg PO once daily	

APPENDIX A. Antidiabetic Agents$^{2,66-69}$, continued

CLASS	AGENT	MECHANISM	SIDE EFFECTS	DOSAGE RANGE AND ROUTE OF ADMINISTRATION	LABORATORY TESTS FOR MONITORING
Amylin analog	Pramlintide (Symlin)	Improves glycemic control by slowing the rate of gastric emptying, preventing postprandial rise in glucagon levels, and increasing sensations of satiety, thereby reducing caloric intake and potentiating weight loss	Nausea, headache, vomiting, anorexia, weight loss	Subcutaneous injection; preprandial, rapid-acting, short-acting, and fixed-mix insulin doses should be decreased by 50% when pramlintide is initiated; for type 1 patients, initial recommended dosing is 15 mcg subcutaneously immediately prior to major meals, which should be titrated at 15-mcg increments to a maintenance dose of 30 mcg or 60 mcg as tolerated; for type 2 patients, initial dose should be 60 mcg subcutaneously prior to each major meal, with a single increment increase to 120 mcg as tolerated	Directly affects PPG; FPG and A1c should be monitored to assess overall glycemic control
Bile acid sequestrants	Colesevelam (Welchol)	Binds bile acids, cholesterol	Constipation, ↑ triglycerides; may interfere with the absorption of other medications	1875 mg (three 625-mg tablets) PO BID or 3750 (six 625-mg tablets) PO once a day	FPG, A1c, triglycerides, LDL, HDL
Dopamine agonists	Bromocriptine (Cycloset)	Increases dopamine levels; inhibits excessive sympathetic tone within the CNS; this enhances suppression of hepatic glucose production and reduces postmeal plasma glucose levels	Somnolence, hypotension, syncope, nausea, vomiting, fatigue	0.8 mg PO once a day in the morning; may increase dose each week with a max dose of 4.8 mg/day	Directly affects PPG; monitor FPG and A1c to assess glycemic control

A1c = glycosylated hemoglobin; ACE = angiotensin converting enzyme inhibitor; ALT = alanine aminotransferase; ARB = angiotensin II receptor blocker; AST = aspartate aminotransferase; ATP-K = adenosine triphosphate–potassium; BID = twice a day; CNS = central nervous system; CrCl = creatinine clearance; DPP-4 = dipeptidyl peptidase-4; eGFR = estimated glomerular filtration rate; FPG = fasting plasma glucose; GI = gastrointestinal; GLP-1 = glucagon-like peptide-1; HDL = high density lipoprotein; hr = hour/s; LDL = low density lipoprotein; max = maximum; min = minute/s; mo = month/s; MEN2 = multiple endocrine neoplasia type 2; MTC = medullary thyroid carcinoma; NYHA = New York Heart Association; PO = by mouth, oral; PPAR = peroxisome proliferator-activated receptor; PPG = postprandial glucose; REMS = risk evaluation and mitigation strategy; SCr = serum creatinine; SGLT2 = sodium–glucose cotransporter 2; TID = three times a day; ULN = upper limit of normal; yr = year/s.

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QUICKVIEW | Total Serum T_3

PARAMETER	DESCRIPTION	COMMENTS
Common reference ranges		
Adults and children	80–200 ng/dL	Affected by TBG changes
	(1.2–3.1 nmol/L)	SI conversion factor = 0.0154 (nmol/L)
Critical value	Not established	Extremely high or low values should be reported quickly
Natural substance?	Yes	Only 0.2% of T_3 is unbound
Inherent activity?	Only free portion	Total assumed to correlate with free T_3 activity
Location		
Production and storage	20–25% secreted by thyroid gland, remainder produced by conversion of T_4 to T_3	Bound mostly to thyroglobulin
Secretion/excretion	From thyroid, liver, and kidneys to blood	
Major causes of…		
High results	Hyperthyroidism	Not truly a cause but a reflection of high result
	T_4 and T_3 supplements	
	Other causes (Table 10-13)	
Associated signs and symptoms	Signs and symptoms of hyperthyroidism	Nervousness, weight loss, heat intolerance, tachycardia, diaphoresis
Low results	Hypothyroidism	Not truly a cause but a reflection of low result
	Other causes (Table 10-13)	
	Propranolol	
	Propylthiouracil	
	Glucocorticoids	
Associated signs and symptoms	Signs and symptoms of hypothyroidism	Lethargy, constipation, dry skin, cold intolerance, slow speech, confusion
After insult, time to…		
Initial elevation or depression	Weeks to months	Increases within hours in acute T_4 or T_3 overdose
Peak values	Weeks to months	Increases within hours in acute T_4 or T_3 overdose
Normalization	Usually same time as onset	Assumes insult removed or effectively treated
Drugs often monitored with test	T_4 and T_3	Other drugs
Causes of spurious results	Increased or decreased TBG leads to falsely increased or decreased total serum T_3, respectively; nonthyroidal illness leads to falsely increased or decreased total serum T_3	Factors affecting TBG (Table 10-10)

T_3 = triiodothyronine; T_4 = thyroxine; TBG = thyroxine-binding globulin; SI = International System of Units

QUICKVIEW | Total Serum T_4

PARAMETER	DESCRIPTION	COMMENTS
Common reference ranges		
Adults and children	5.5–12.5 mcg/dL (71–161 nmol/L)	Affected by TBG changes with nonthyroidal illness
		SI conversion factor = 12.87 (nmol/L)
Newborn/3–5 days	11–23/9–18 mcg/dL	Affected by TBG changes with nonthyroidal illness
Critical value	Not established	Extremely high or low values should be reported quickly, especially in newborns
Natural substance?	Yes	Only 0.02% of T_4 is unbound
Inherent activity?	Only free portion	Total assumed to correlate with free T_4 activity
Location		
Production and storage	Thyroid gland	Bound mostly to thyroglobulin
Secretion/excretion	From thyroid to blood	About 33% converted to T_3 outside thyroid
Major causes of…		
High results	Hyperthyroidism	Not truly a cause but a reflection of high result
	T_4 supplements	
	Other causes (Table 10-6 and Table 10-8)	
Associated signs and symptoms	Signs and symptoms of hyperthyroidism	Nervousness, weight loss, heat intolerance, tachycardia, diaphoresis
Low results	Hypothyroidism	Not truly a cause but a reflection of low result
	Other causes (Table 10-5 and Table 10-8)	
	Signs and symptoms of hyperthyroidism	Lethargy, constipation, dry skin, cold intolerance, slow speech, confusion
After insult, time to…		
Initial elevation or depression	Weeks to months	Increases within hours in acute T_4 overdose
Peak values	Weeks to months	Increases within hours in acute T_4 overdose
Normalization	Usually same time as onset	Assumes insult removed or effectively treated
Drugs often monitored with test	T_4	Other drugs
Causes of spurious results	Increased or decreased TBG leads to falsely increased or decreased total serum T_4, respectively; nonthyroidal illness leads to falsely increased or decreased total serum T_4	Factors affecting TBG (Table 10-13)

T_3 = triiodothyronine; T_4 = thyroxine; TBG = thyroxine-binding globulin; SI = International System of Units.

QUICKVIEW | Free T_4

PARAMETER	DESCRIPTION	COMMENTS
Common reference ranges		
Adults and children	0.9–2.3 ng/dL (11.6–29.6 pmol/L)	Higher in infants <1 mo; direct equilibrium dialysis assay not affected by TBG changes or severe nonthyroidal illness SI conversion factor = 12.87 (pmol/L)
Critical value	Not established	Extremely high or low values should be reported quickly
Natural substance?	Yes	Only 0.02% of T_4 is unbound
Inherent activity?	Probably	Some influence on basal metabolic rate; T_3 most active
Location		
Production and storage	Thyroid gland	Bound mostly to thyroglobulin
Secretion/excretion	From thyroid to blood	33% converted to T_3 outside thyroid
Major causes of…		
High results	Hyperthyroidism T_4 supplements Other causes (Table 10-8)	Not truly a cause but a reflection of high result
Associated signs and symptoms	Signs and symptoms of hyperthyroidism	Nervousness, weight loss, heat intolerance, tachycardia, diaphoresis
Low results	Hypothyroidism Other causes (Table 10-8)	Not truly a cause but a reflection of low result
Associated signs and symptoms	Signs and symptoms of hypothyroidism	Lethargy, constipation, dry skin, cold intolerance, slow speech, confusion
After insult, time to…		
Initial elevation or depression	Weeks to months	Increases within hours in acute T_4 overdose
Peak values	Weeks to months	Increases within hours in acute T_4 overdose
Normalization	Usually same time as onset	Assumes insult removed or effectively treated
Drugs often monitored with test	T_4	Other drugs
Causes of spurious results	Rare with direct equilibrium dialysis assay (Table 10-9)	Decreased direct equilibrium dialysis assay for free T_4 with decreased or normal TSH may occur in patients

mo = month; T_3 = triiodothyronine; T_4 = thyroxine; TSH = thyroid-stimulating hormone.

QUICKVIEW | TSH

PARAMETER	DESCRIPTION	COMMENTS
Common reference ranges		
Adults and children	0.5–5.0 milliunits/L	
Critical value	Not established	Extremely high or low values should be reported quickly
Natural substance?	Yes	
Inherent activity?	Yes	Stimulates thyroid to secrete hormone
Location		
Production and storage	Anterior pituitary	
Secretion/excretion	Unknown	
Major causes of…		
High results	Primary hypothyroidism	Causes of primary hypothyroidism
	Antithyroid drugs	
	Other causes (Table 10-6)	
Associated signs and symptoms	Signs and symptoms of hypothyroidism	Lethargy, constipation, dry skin, cold intolerance, slow speech, confusion
Low results	Primary hyperthyroidism	Must be ≤0.05 milliunit/L for definitive diagnosis of (primary hyperthyroidism); may be decreased or normal in secondary or tertiary hypothyroidism
	Other causes (Table 10-5 and Table 10-8)	
Associated signs and symptoms	Signs and symptoms of hyperthyroidism	Nervousness, weight loss, heat intolerance, HR increase, diaphoresis
After insult, time to…		
Initial elevation or depression	Weeks to months	Decreases within hours in acute T_4 overdose
Peak values	Weeks to months	Decreases within hours in acute T_4 overdose
Normalization	Usually same time as onset	Assumes insult removed or effectively treated
Drugs often monitored with test	T_4 and T_3	Also antithyroid drugs (methimazole and propylthiouracil)
Causes of spurious results	Increased TSH: dopamine antagonists	Metoclopramide and domperidone
	Decreased TSH: dopamine agonists	Dopamine, bromocriptine, levodopa, glucocorticoids
	Above TSH measurements are accurate here	These drugs decrease TSH, but the change is not reflective of primary hypothyroidism or hyperthyroidism; therefore, the results are not truly spurious

HR = heart rate; T_3 = triiodothyronine; T_4 = thyroxine; TSH = thyroid-stimulating hormone.

QUICKVIEW | Plasma Glucose

PARAMETER	DESCRIPTION	COMMENTS
Common reference ranges		
Adults	Adult fasting: <100 mg/dL (5.6 mmol/L)	Multiply by 0.056 for SI units (mmol/L)
	Adult 2-hr postprandial: <140 mg/dL (7.8 mmol/L)	
	Full-term infant normal: 20–90 mg/dL (1.11–5 mmol/L)	
Critical value	Adult fasting, no previous history: >126 mg/dL (7 mmol/L)	In known patient with diabetes, increased glucose is not an immediate concern unless patient is symptomatic; an increased glucose is not critical if serial levels are decreasing over time
	Anytime: <50 mg/dL (<3.9 mmol/L)	
Natural substance?	Yes	Always present in blood
Inherent activity?	Yes	Major source of energy for cellular metabolism
Location		
Production	Liver and muscle	Dietary intake
Storage	Liver and muscle	As glycogen
Secretion/excretion	Mostly metabolized for energy	Levels >180 mg/dL (10 mmol/L) spill into urine
Major causes of…		
High results	Type 1 and 2 DM	
	Drugs (Table 10-2)	
	Excess intake	
Associated signs and symptoms	Polyuria, polydipsia, polyphagia, weakness	Long term: damage to kidneys, retina, neurons, and vessels
Low results	Insulin secretion/dose excessive relative to diet	Most common in diabetics
	Sulfonylureas or other hypoglycemic agents	
	Insulinomas	
Associated signs and symptoms	Hunger, sweating, weakness, trembling, headache, confusion, seizures, coma	From neuroglycopenia and adrenergic discharge
After insult, time to…		
Initial elevation	Type 1 DM: months to elevation	
	Type 2 DM: years to elevation	
	After insulin: minutes to decrease	
	After meal: 15–30 min to elevation	
	After epinephrine or glucagon: minutes	
	After steroids and growth hormone: hours	
Normalization	After insulin: minutes	Depends on insulin type
	After exercise: minutes to hours	Depends on intensity and duration
Drugs often monitored with test	Insulin, sulfonylureas, biguanides (e.g., metformin), thiazolidinediones, or other hypoglycemic agents	Also, diazoxide, L-asparaginase, total parenteral nutrition
Causes of spurious results	High-dose vitamin C	With some glucometers
	Metronidazole	With some automated assays

DM = diabetes mellitus; min = minutes; SI = International System of Units.

QUICKVIEW | A1c

PARAMETER	DESCRIPTION	COMMENTS
Common reference ranges		
Adults and children	4–5.6%	Fasting not required; represents average glucose levels past 8 wk
Critical value	Not applicable	Reflects long-term glycemic control; >9% suggests poor control
Natural substance?	Yes	Subunit of Hgb
Inherent activity?	Yes	Oxygen carrier; also carries glucose
Location		
Production	Bone marrow	In newborns in liver and spleen
Storage	Not stored	Circulates in blood
Secretion/excretion	Older cell removed by spleen	Converted to bilirubin
Major causes of…		
High results	DM	Any cause of prolonged hyperglycemia
	Chronic hyperglycemia	
Associated signs and symptoms	Signs and symptoms of diabetes	
Low results		
Associated signs and symptoms	Not clinically useful	
After insult, time to…		
Initial elevation	2–4 mo	Initial insult is chronic hyperglycemia
Normalization	2–4 mo	Assumes sudden and persistent euglycemia
Drugs often monitored with test	Insulin, sulfonylureas, biguanides	Also diet and exercise
Causes of spurious results	High results: alcoholism, uremia, increased triglycerides, hypertriglyceridemia, hemolysis, polycythemia Low results: sickle cell anemia, thalassemia	Also seen in pregnant and splenectomized patients

DM = diabetes mellitus; Hgb = hemoglobin; mo = months; wk = weeks.

11

THE KIDNEYS

Dominick P. Trombetta

OBJECTIVES

After completing this chapter, the reader should be able to

- Describe the normal physiology of the kidneys
- Differentiate the renal handling of urea and creatinine
- Describe clinical situations where blood urea nitrogen and serum creatinine are elevated
- Describe the evolving role of cystatin C in estimating glomerular filtration rate
- Describe the limitations in the usefulness of the serum creatinine concentration in estimating kidney function
- Understand the clinical utility of the Cockcroft-Gault equation, the Modification of Diet in Renal Disease equation, and the Chronic Kidney Disease Epidemiology Collaboration equations to assess kidney function
- Determine creatinine clearance given a patient's 24-hour urine creatinine excretion and serum creatinine
- Estimate creatinine clearance given a patient's height, weight, sex, age, and serum creatinine and identify limitations of the methods for estimation of kidney function
- Understand the classification of chronic kidney disease, glomerular filtration rate categories, and albuminuria as predictors of disease

(continued on page 238)

Through the excretion of water and solutes, the kidneys are responsible in large part for maintaining homeostasis within the body. They also function in the activation and synthesis of many substances that affect blood pressure (BP), mineral metabolism, and red blood cell (RBC) production. The purpose of this chapter is to provide insight to the interpretation of laboratory tests in the assessment of kidney function, as well as provide an overview of the interpretation of a urinalysis.

KIDNEY PHYSIOLOGY

The functional unit of the kidneys is the nephron (**Figure 11-1**), and each of the two kidneys contains about 1 million nephrons. The major components of the nephron include the glomerulus, proximal tubule, loop of Henle, distal tubule, and collecting duct. Blood is delivered to the glomerulus, the filtering portion of the nephron, via the afferent arteriole. Acting as microfilters, the pores of glomerular capillaries allow substances with a molecular weight of up to 40,000 daltons to pass through them. Plasma proteins, such as albumin (mw 65,000 daltons) and RBCs do not normally pass through the glomerulus. Ionic charge also affects filtration as the glomerulus selectively retains negatively charged proteins such as albumin. In kidney disease involving the glomerulus, the effect of ionic charge becomes less discriminate and albuminuria develops. Most drugs are small enough to be freely filtered at the glomerulus, with the exception of large proteins and drugs bound to plasma proteins.[1]

The proximal tubule reabsorbs large quantities of water and solute. Sodium passively follows the reabsorption of water back into the blood. Glucose, uric acid, chloride, bicarbonate, amino acids, urea, hydrogen, phosphate, calcium, and magnesium also are primarily reabsorbed by the proximal tubule. Sodium, chloride, magnesium, and water are further reabsorbed in the loop of Henle. The distal tubule controls the amounts of sodium, potassium, bicarbonate, phosphate, and hydrogen that are excreted, and the collecting duct regulates the amount of water in the urine as a result of the effect of antidiuretic hormone (ADH), which facilitates water reabsorption.[1]

As shown in Figure 11-1, substances can enter the nephron from the peritubular blood or interstitial space via secretion. In addition, substances can be reabsorbed from primarily the distal tubule back into the systemic circulation via the peritubular vasculature. Tubular secretion occurs via two primary pathways in the proximal tubule: the organic acid transport system and the organic cation transport (OCT) system. Although each system is somewhat specific for anions and cations, respectively, some drugs such as probenecid are secreted by both pathways. Creatinine enters the tubule primarily by filtration through the glomerulus. However, a small amount of creatinine is also secreted by the OCT system into the proximal tubule. This becomes important when using the renal clearance of creatinine to estimate kidney function.[1]

Blood flow to the kidneys is determined, in large part, by cardiac output with about 20% or 1.2 L/min directed to the kidneys. Renal plasma flow (RPF) is directly related to renal blood flow (RBF) by taking the patient's hematocrit into consideration as follows:

$$\text{RPF} = \text{RBF} \times (1 - \text{Hct}) \qquad (1)$$

OBJECTIVES

- **Describe the role of the pharmacist in the care of patients with acute or chronic kidney disease**
- **Discuss the various components assessed by macroscopic, microscopic, and chemical analysis of the urine**
- **Describe the role of commonly obtained urinary electrolytes and the fractional excretion of sodium in the diagnostic process**

where RPF = renal plasma flow; RBF = renal blood flow; and Hct = hematocrit. The normal value for RPF is about 625 mL/min. Of the plasma that reaches the glomerulus, about 20% is filtered and enters the proximal tubule, resulting in a glomerular filtration rate (GFR) of about 125 mL/min. The GFR is often used as a measure of the degree of kidney excretory function in a patient. The kidneys filter about 180 L of fluid each day; of this amount, they excrete only 1.5 L as urine. Thus, more than 99% of the initial glomerular filtrate is reabsorbed back into the bloodstream. Many solutes, such as creatinine and many renally eliminated drugs, are concentrated in the urine.[1]

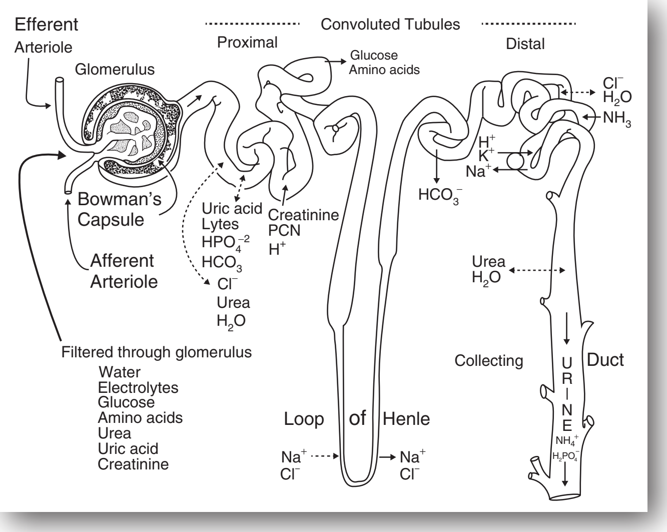

FIGURE 11-1. The nephron. Arrows pointing toward the nephron represent substances entering from the peritubular blood or interstitial space. Arrows heading away represent reabsorption. Solid arrows represent an active (energy-requiring) process, and dashed arrows represent a passive process. PCN = penicillin; lytes = electrolytes. *Source*: Reprinted from Inker LA, Astor BC, Fox CH et al. KDOQI US commentary on the 2012 KDIGO clinical practice guideline for the evaluation and management of CKD. *Am J Kidney Dis.* 2014; 63:713-35. Copyright ©2014, with permission from Elsevier.

DEFINITION AND CLASSIFICATION OF CHRONIC KIDNEY DISEASE

The classification of ***chronic kidney disease*** (CKD) is based on the nature or cause of the abnormality (structure, function), the GFR category (g1 through g5), and albuminuria category (a1 through a3). The prognostic categories can be found at http://www.kdigo.org/clinical_practice_guidelines/pdf/CKD/KDIGO_2012_CKD_GL.pdf.[2] CKD has been defined as GFR <60 mL/min/1.73 m^2 for greater than three months.[2] The use of estimating equations (eGFR) or the measurement of GFR rather than using serum creatinine (SCr) or cystatin C alone can provide the basis for the classification of kidney function. Additionally, markers of kidney damage such as albuminuria (albumin excretion rate >30 mg/24 hr, albumin-to-creatinine [ACR] ratio 30 mg/g), structural (e.g., polycystic kidney disease, hydronephrosis, renal artery stenosis), or functional anomalies (e.g. urinary sediment such as casts or renal tubular disorders) are used for prognosis of risk: low risk (no markers of kidney disease), moderately increased risk, high risk, and very high risk.[2] This information is useful to guide therapy and further monitoring of and CKD complications.

ASSESSMENT OF KIDNEY FUNCTION

Classification of kidney disease as well as dosing of medications depends on an accurate and reliable method of assessing kidney function.[2] Direct measurement of GFR using markers such as inulin and iothalamate is the most accurate assessment of kidney function but is not used routinely in clinical practice due to cost and practical concerns. Measurements of timed 24-hour urine creatinine (UCr) collections are difficult by design, flawed by collection errors, and used only when determination of GFR is vital and the use of the eGFR equations are not reliable. The estimation of creatinine clearance (CrCl) through equations such as Cockcroft-Gault has been the "gold standard" for drug dosing. Recently, serum concentrations of cystatin C, an endogenous amino acid, have been evaluated as an alternative method to predict GFR in children as well as adults.[3-5] eGFR had been initially validated using the Modification of Diet in Renal Disease (MDRD) equation and was used to stage and monitor CKD until recently.[6-10] The CKD Epidemiology Creatinine Equation 2009 is now currently recommended to estimate GFR.[2] Recently, the U.S. Food and Drug Administration (FDA) Guidance to Industry draft revision has proposed that both the eGFR and CrCl be incorporated into the package insert dosage recommendations for patients with decreased renal function.[11] With more clinical laboratories reporting eGFR values and as pharmacokinetic data reference both eGFR and CrCl for new medications, the use of eGFR to adjust medication doses may become more commonplace.

Exogenous Markers

Inulin Clearance

Normal range[12]: men = 125 + 15 (SD) mL/min/m^2; women = 110 + 15 mL/min/m^2

Inulin is a fructose polysaccharide, an inert carbohydrate, with a molecular weight of 5200 daltons, which is not bound to plasma proteins. Inulin is freely filtered through the glomerulus and not metabolized, secreted, or reabsorbed and can be regarded as the gold standard for measuring GFR in adults and older children.[13] Neonates and younger children may present logistical problems in obtaining accurate urine flow rates.[9] This test is fairly invasive because inulin must be administered intravenously, and it requires special analytical methods that limit its practical application in many healthcare facilities.[9]

I-Iothalamate and Cr-EDTA Clearance

Normal reference range[14] = 87–141 mL/min/SA

The urinary clearance of the radioactive marker *I-Iothalamate* is being more commonly used in research settings. The test involves injection of the radioactive exogenous marker, repeated blood sampling, and timed urine collection. The invasiveness and associated costs prohibit widespread application. The administration of ^{51}Cr-EDTA (ethylenediamine tetra-acetic) has been used in the past as an alternative to I-Iothalamate. As with inulin, the need for intravenous (IV) administration and timed urine collections for both these markers make them impractical for routine use.

Endogenous Markers

Cystatin C

Normal reference range[15]: men, 0.6–1.52 mg/L; women, 0.57–1.45 mg/L

Cystatin C is a protease inhibitor produced at a steady state by all nucleated cells. It is filtered by the glomerulus and neither reabsorbed or secreted by the kidney.[16] It had been originally proposed that cystatin C may be more sensitive than SCr in tracking changes in kidney function and that it is unaffected by diet or muscle mass. Serum cystatin C concentrations would be expected to be inversely proportional to GFR. Changes in serum cystatin C concentrations may be an indirect reflection of GFR. Combining SCr with cystatin C, age, sex, and race in estimating GFR have provided better results than equations based on a single filtration marker.[17] Cystatin C concentrations have been used in equations for estimating GFR in pediatric patients.[3] The use of certified cystatin C concentrations in eGFR equations is evolving rapidly with the recent certification of reference material.[18] The National Kidney Disease Education Program (NKDEP) recommends the use of cystatin C equations derived from data with the ERM-DA471/FCC reference material that had been certified. Currently, it is recommended that inclusion of traceable cystatin C be used in eGFR equations rather than relying on the absolute value in the assessment of CKD.[2] Data also support the correlation of elevated cystatin C levels and cardiovascular disease mortality.[19]

Serum Creatinine

Normal range: adults, 0.6–1.2 mg/dL (53–106 μmol/L); young children, 0.2–0.7 mg/dL (18–62 μmol/L)

Creatinine and its precursor creatine are nonprotein, nitrogenous biochemicals of the blood. After synthesis in the liver, creatine diffuses into the bloodstream. Creatine then is taken up by muscle cells, where some of it is stored in a high-energy form, creatine phosphate. Creatine phosphate acts as a readily available source of phosphorus for regeneration of adenosine triphosphate and is required for transforming chemical energy to muscle action.

Creatinine, which is produced in the muscle, is a spontaneous decomposition product of creatine and creatine phosphate. The daily production of creatinine is about 2% of total body creatine, which remains constant if muscle mass is not significantly changed. In normal patients at steady state, the rate of creatinine production equals its excretion. Therefore, SCr concentrations vary little from day-to-day in patients with healthy kidneys. Although there is an inverse relationship between SCr and kidney function, SCr should not be the sole basis for the evaluation of renal function.[9] Several issues should be considered when evaluating a patient's SCr. Some of factors that affect SCr concentrations are muscle mass, sex, age, race, medications, method of laboratory analysis, and low-protein diets. Additionally, acute changes in a patient's GFR such as in acute kidney injury may not be initially manifested as an increase in SCr concentration because it takes time for new steady-state concentrations of SCr to be achieved. The time required to reach 95% of steady state in patients with 50%, 25%, and 10% of normal kidney function is about one, two, and four days, respectively. Steady-state concentrations of SCr become very important as they are integral in clinical practice estimations of renal function.

A SCr concentration within the reference ranges as reported by clinical laboratories does not necessarily indicate normal kidney function. For example, a SCr concentration of 1.5 mg/dL in a 45-year-old male who weighs 150 pounds and a 78-year-old female who weighs 92 pounds would correspond to different GFRs.

Clinicians can surmise that as long as no abnormalities exist in muscle mass, an increased SCr almost always reflects a decreased GFR. The converse is not always true; a normal SCr does not necessarily imply a normal GFR. As part of the aging process, both muscle mass and renal function diminish. Therefore, SCr may remain in the normal range because as the kidneys become less capable of filtering and excreting creatinine, they also are presented with decreasing amounts of creatinine. Thus, practitioners should not rely solely on SCr as an index of renal function.

Besides aging and alterations in muscle mass, some pathophysiological changes can affect the relationship between SCr and kidney function. For example, renal function may be overestimated on the basis of SCr alone in cirrhotic patients. In this patient population, the low SCr is due to a decreased hepatic synthesis of creatine, the precursor of creatinine. In cirrhotic patients, it is prudent to perform a measured 24-hour CrCl. If the patient also has hyperbilirubinemia, assay interference by elevated bilirubin also may contribute to a low SCr.

Laboratory measurement and reporting of SCr. Historically, the laboratory methods used to measure SCr included the alkaline picrate method, inorganic enzymatic methods, and high-pressure liquid chromatography. The alkaline picrate assay (Jaffe) was the most commonly used method to measure SCr; however, interfering substances such as noncreatinine chromogens can often lead to underestimation of kidney function. Causes of falsely elevated SCr results included unusually large amounts of noncreatinine chromogens (e.g., uric acid, glucose, fructose, acetone, acetoacetate, pyruvic acid, and ascorbic acid) in the serum. For example, an increase in glucose of 100 mg/dL (5.6 mmol/L) could falsely elevate SCr by 0.5 mg/dL (44 μmol/L) in some assays. Likewise, serum ketones high enough to spill into the urine may falsely increase SCr and UCr. In patients in diabetic ketoacidosis (DKA), false elevation could precipitate unnecessary evaluation for renal failure when presenting with ketoacidosis. Like ketones, acetoacetate may have been elevated enough to cause a falsely elevated SCr after a 48-hour fast or in patients with DKA. Another endogenous substance, bilirubin, can falsely lower SCr results with both the alkaline picrate and enzymatic assays. At low GFRs, however, creatinine secretion overtakes the balancing effects of measuring noncreatinine chromogens, causing an overestimation of GFRs by as much as 50%.[5]

Reliable and accurate measurement and subsequent reporting of SCr concentrations is very important. The MDRD equation utilizes as one of its variables the SCr concentration to stage kidney damage based on the estimation of GFR. The Cockcroft-Gault equation, which is highly dependent on the SCr concentration, has been used as the accepted methodology for drug dosing based on an estimation of CrCl.[20] The greater the imprecision of the assay, the less accurate the resultant GFR estimations. The primary source of measurement errors includes systematic bias and interlaboratory, intralaboratory, and random variability in daily calibration of SCr values. Interlaboratory commutability is also problematic secondary to the variations in assay methodologies. Recently, a report from the Laboratory Working Group of the National Kidney Disease Program, made recommendations to improve and standardize measurement of SCr.[21,22] As of 2011, creatinine standardization is reported to be nationwide (in the United States) and calibration should be traceable to isotope dilution mass spectrometry (IDMS). Of note, SCr concentrations are lower than had been previously reported with older methods. Calculations of renal function using Cockcroft-Gault or other recommended creatinine-based equations should use IDMS traceable measured SCr.

Urea (Blood Urea Nitrogen)

Normal range: 8–23 mg/dL (2.9–8.2 mmol/L)

Blood urea nitrogen (BUN) is actually the concentration of nitrogen (as urea) in the serum and not in RBCs as the name implies. Although the renal clearance of urea can be measured, it cannot be used by itself to assess kidney function. Its serum concentration depends on urea production (which occurs in the liver), glomerular filtration, and tubular reabsorption. Therefore, clinicians must consider factors other than filtration when interpreting changes in BUN.

When viewed with other laboratory and clinical data, BUN can be used to assess or monitor hydration, renal function, protein tolerance, and catabolism in numerous clinical settings (**Table 11-1**).[23] Also, it is used to predict the risk of uremic syndrome in patients with severe renal failure. Concentrations above 100 mg/dL (35.7 mmol/L) are associated with this risk.

Elevated BUN

Urea production is increased by a high-protein diet (including amino acid infusions), upper gastrointestinal (GI) bleeding, and administration of corticosteroids, tetracyclines, or any other drug with antianabolic effects. Usually, about 50% of the filtered urea is reabsorbed, but this amount is inversely related to the rate of urine flow in the tubules. In other words, the slower the urine flows, the more time the urea has to leave the tubule and re-enter surrounding capillaries (reabsorption). Urea reabsorption tends to change in parallel with sodium, chloride, and water reabsorption. Because patients with volume depletion avidly reabsorb sodium, chloride, and water, larger amounts of urea are also absorbed.

Likewise, a patient with a pathologically low BP may develop diminished urine flow secondary to decreased RBF with a subsequently diminished GFR. Congestive heart failure and reduced RBF, despite increased intravascular volume, is a common cause of elevated BUN. Types of renal failure that can cause an abnormally high BUN (also called *azotemia*) are listed in Table 11-1.

Decreased BUN

In and of itself, a low BUN does not have pathophysiological consequences. BUN may be low in patients who are malnourished or have profound liver damage (due to an inability to synthesize urea). Intravascular fluid overload may initially dilute BUN (causing low concentrations), but many causes

TABLE 11-1. Common Causes of True BUN Elevations (Azotemia)

Prerenal causes
Decreased renal perfusion: dehydration, blood loss, shock, severe heart failure
Intrarenal (intrinsic) causes
Acute kidney failure: nephrotoxic drugs, severe hypertension, glomerulonephritis, tubular necrosis
Chronic kidney dysfunction: pyelonephritis, diabetes, glomerulonephritis, renal tubular disease, amyloidosis, arteriosclerosis, collagen vascular disease, polycystic kidney disease, overuse of NSAIDs
Postrenal causes
Obstruction of ureter, bladder neck, or urethra

BUN = blood urea nitrogen; NSAIDs = nonsteroidal anti-inflammatory drugs.

of extravascular volume overload, which are associated with third spacing of fluids into tissues (e.g., congestive heart failure, renal failure, and nephrotic syndrome) result in increased BUN because effective circulating volume is decreased.

BUN to SCr Ratio

Simultaneous BUN and SCr determinations are commonly made and can furnish valuable information to assess kidney function. This is particularly true for acute kidney injury. In acute kidney injury due to volume depletion, both BUN and SCr are elevated. However, the BUN:SCr ratio is often >20:1). This observation is due to the differences in the renal handling of urea and creatinine. Recall that urea is reabsorbed with water, and under conditions of decreased renal perfusion both urea and water reabsorption are increased. Because creatinine is not reabsorbed, it is not affected by increased water reabsorption. So the concentrations of both substances may increase in this setting, but the BUN would be increased to a greater degree, leading to a BUN:SCr >20:1.

In summary, when acute changes in kidney function are observed, and both BUN and SCr are greater than normal limits, BUN:SCr ratios >20:1 suggest prerenal causes of acute renal impairment (Table 11-1), whereas ratios from 10:1 to 20:1 suggest intrinsic kidney damage. Furthermore, a ratio >20:1 is not clinically important if both SCr and BUN are within normal limits (e.g., SCr = 0.8 mg/dL and BUN = 20 mg/dL).

Measurement of CrCl

A complete 24-hour urine collection to measure CrCl is best in clinical situations of unstable SCr.[24] In addition, the National Kidney Foundation indicates that these measured CrCls are not better than the estimates of CrCl provided through recommended equations.[2] However, a 24-hour, timed urine measurement of CrCl may be useful in the following clinical situations: patients starting dialysis; in the presence of acute changes in kidney function in hospitalized patients (e.g., acute kidney injury, acute renal failure) or those with comorbid medical conditions; during evaluation of dietary intake; patients with extremes in muscle mass; health enthusiasts taking creatine supplementation; vegetarians; patients with quadriplegia or paraplegia; and patients who have undergone amputations.[2]

Interpreting CrCl Values with Other Renal Parameters

As noted previously, the most common clinical uses for CrCl and SCr are below:

- Assessing kidney function in patients with CKD
- Monitoring the effects of drug therapy on slowing the progression of kidney disease
- Monitoring patients on nephrotoxic drugs
- Determining dosage adjustments for renally eliminated drugs

Because the relationship between SCr and CrCl is inverse and geometric as opposed to linear (**Figure 11-2**), significant declines in CrCl may occur before SCr rises above the normal range. For example, as CrCl slows, SCr rises very little until there is a significant reduction in renal function. Therefore, SCr alone is not a sensitive indicator of early kidney dysfunction.

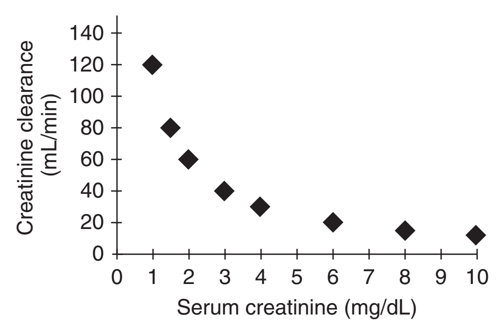

FIGURE 11-2. This plot represents the inverse relationship between SCr and CrCl. Relatively small changes in SCr at lower levels represent significant change in kidney function as assessed by CrCl.

Calculating CrCl from a Timed Urine Collection

Although shorter collection periods (three to eight hours) appear to be adequate and may be more reliable, CrCl is routinely calculated using a 12-hour or 24-hour urine collection. Creatinine excretion is normally 20–28 mg/kg/24 hr in men and 15–21 mg/kg/24 hr in women. In children, normal excretion (mg/kg/24 hr) should be approximately 15 + (0.5 × age), where age is in years.

Because its excretion remains relatively consistent within these ranges, UCr is often used as a check for the completion of the urine collection. In adults, some clinicians discount a urine sample if it contains <10 mg of creatinine/kg/24 hr and assume that the collection was incomplete. However, 8.5 mg/kg/day might be a better cutoff, especially in critically ill elderly patients. UCr assays are affected by most of the same substances that affect SCr. To interfere significantly, however, the substance must appear in the urine in concentrations at least equal to those found in the blood.

Measured CrCl is calculated using the following formula:

$$\text{CrCl (mL/min)} = [\text{UCr} \times \text{V}]/[\text{SCr} \times \text{T}] \times \frac{1.73}{\text{BSA}} \tag{2}$$

where CrCl is the CrCl in mL/min/1.73 m^2; UCr = urine creatinine concentration (mg/dL); V = volume of urine produced during the collection interval (mL); SCr = serum creatinine concentration (mg/dL); T = time of the collection interval (minutes), and BSA = body surface area (m^2).

BSA can be estimated using the standard method of Dubois and Dubois:

$$\text{BSA (m}^2\text{)} = 0.20247 \times \text{height (m)}^{0.725} \times \text{weight (kg)}^{0.425} \tag{3}$$

BSA also can be estimated using the following equations from Mosteller[25]:

$$\text{BSA (m}^2\text{)} = \sqrt{[\textit{height (cm)} \times \textit{weight (kg)}]/3600} \tag{4}$$

$$\text{BSA (m}^2\text{)} = \sqrt{[\textit{height (in)} \times \textit{weight (lb)}]/3131} \tag{5}$$

Adjustment of CrCl to a standard BSA (1.73 m^2) allows direct comparison with normal CrCl ranges because such tables are in units of milliliters per minute per 1.73 m^2. The CrCl value adjusted for BSA is the number of milliliters cleared per minute for each 1.73 m^2 of the patient's BSA. Therefore, such adjustment in a large person (>1.73 m^2) reduces the original nonadjusted clearance value because the assumption is that clearance would be lower if the patient were smaller. In practice, it is only important to adjust CrCl for BSA in patients who are much smaller or larger than 1.73 m^2.

Estimation of CrCl

In practice, dosage recommendations for medications excreted through the kidneys have been traditionally based on the Cockcroft-Gault estimation of CrCl. With the implementation of standardized reporting of creatinine values, calculated CrCl values may be 5–20% higher and may not correlate with dosage guidelines based on renal dose adjustments on creatinine values from older assays.

Cockcroft-Gault Equation

This formula provides an estimation of CrCl.[20] The patient's age, total body weight (TBW), and SCr concentration are necessary for the estimation. There is some controversy regarding which patient weight to use in the formula. Few patients are close to their ideal body weight (IBW). There are weight adjustment formulas that have empirically attempted to improve the estimation of CrCl by calculating an adjusted body weight. Additional attempts to improve the Cockcroft-Gault equation such as rounding SCr to 1 and using adjusted or lean body weight have all been attempted without clinical validation. This equation should be used cautiously in patients with unstable renal function.

$$\underset{(\text{mL/min})}{\text{CrCl}} = \frac{(140 - \text{age}) \times \text{weight (kg)}}{72 \times \text{SCr (mg/dL)}} \times 0.85 \text{ (if female)} \qquad (6)$$

$$\underset{(\text{mL/min})}{\text{CrCl}} = \frac{(140 - \text{age}) \times \text{weight (kg)}}{72 \times \text{SCr (mg/dL)}} \times \underset{(\text{if female})}{0.85} \times \frac{1.73 \text{ m}^2}{\text{BSA}} \qquad (7)$$

Cockcroft-Gault IBW, where IBW (kg) =
(2.3 × inches >5 feet) + 50 (if male),
or (2.3 × inches >5 feet) + 45.5 (if female)

Cockcroft-Gault TBW = TBW for weight in CG equation

Pediatric Patients

The 2012 National Kidney Foundation Kidney Disease Outcome Quality Initiative (NKF KDOQI) Clinical Practice Guidelines for the Evaluation and Management of CKD do not provide specific guidance regarding which equation to use in pediatric patients.[2] However, NKDEP recommends the bedside IDMS-traceable Schwartz equation.[26] The Schwartz equation provides an estimation of CrCl whereas the Counahan-Barratt equation provides an estimation of the GFR. The older version of the Schwartz equation was derived from inulin clearance and measured creatinine concentrations, which could overestimate the true value.[27,28] The Schwartz equation has been recently modified to integrate the newer standardized SCr values.[29,30] The bedside formula may be more practical because the majority of clinical laboratories may not be able to report cystatin C values in a timely manner. The Counahan-Barratt equation was developed using 51Cr-EDTA plasma clearance.[10] Both equations are imprecise to some degree; however, they represent a more practical alternative than using SCr alone. There is a clear need for a more precise formula for estimating kidney functions in the pediatric population.[4]

Bedside IDMS-traceable Schwartz equation[29]:

$$\text{GFR (mL/min/1.73 m}^2\text{)} = (0.413 \times \text{height (cm)/serum creatinine (mg/dL)} \qquad (8)$$

IDMS-traceable Schwartz equation[29]:

$$\text{GFR (mL/min/1.73 m}^2\text{)} = 39.1\ [(\text{height})\ (\text{m})/\text{SCr (mg/dL)}]^{0.516} \times [(1.8/\text{cystatin C (mg/L)}]^{0.294}\ [30/\text{BUN (mg/dL)}]^{0.169}\ [1.099]^{\text{male}}\ [\text{height (m)}/1.4]^{0.188} \qquad (9)$$

Counahan-Barratt equation[10]:

$$\text{GFR (mL/min/1.73 m}^2\text{)} = 0.43\ [\text{height (cm)/SCr (mg/dL)}] \qquad (10)$$

Estimation of GFR—MDRD Equation

The MDRD equation had been developed as a tool to identify those patients at risk for complications arising from CKD.[9,31] See **Table 11-2** for the stages of CKD. Although the MDRD equation is no longer recommended for use, there may be some laboratories that have not yet updated their reporting of eGFR. The MDRD equation provides an estimated GFR, which was developed using measured GFR I-Iothalamate reference values. Patients at age extremes may be particularly vulnerable to errors of estimated GFR.[4,8] The abbreviated MDRD equation has been re-expressed to include standardized SCr traceable to IDMS values.[6,22,32-34] In addition, results of the MDRD equation should be interpreted cautiously in patients with low muscle mass (e.g., cachectic patients) or those with unstable renal function. One limitation identified with the MDRD equation that is well known is the underestimation of renal function for patients with eGFR >60 mL/min/1.73 m^2.[35] This can lead to more patients being falsely identified with CKD. In these populations, the Kidney Disease: Improving Global Outcomes (KDIGO) working group recommends the measurement of cystatin C or direct measurement of CrCl when SCr concentration is less accurate.[2]

TABLE 11-2. Chronic Kidney Disease Stages[2]

STAGE	GFR (mL/min/1.73 m^2)	INTERPRETATION
1	>90	Normal or high GFR
2	60–89	Mildly decreased
3	A 45–59	Mildly to moderately decreased
	B 30-44	Moderately to severely decreased
4	15–29	Significantly decreased
5	<15	Kidney failure

GFR = glomerular filtration rate.

Abbreviated MDRD equation using revised calibration for SCr:

$$\begin{aligned}\text{GFR (mL/min/1.73 m}^2) &= 175 \times \text{standardized SCr}^{-1.154} \\ &\times \text{age}^{-0.203} \times 1.210 \text{ (if African American)} \\ &\times 0.742 \text{ (if female)}\end{aligned} \tag{11}$$

Estimation of GFR—CKD Epidemiology Collaboration Creatinine Equation 2009

Introduced in 2009, the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation is also based on standardized SCr, age, sex, and race. This equation performs with the same degree of accuracy as the MDRD equation for patients with eGFR <60 mL/min/1.73 m^2.[36,37] However, it corrects the inadequacy of the MDRD equation, which leads to underestimations in those patients with eGFR >60 mL/min/1.73 m^2. Both the CKD-EPI and the MDRD equations account for the age of the patient. Like all SCr-based equations, the Cockcroft-Gault, MDRD, and CKD-EPI equations succumb to the same inherent problems associated with this endogenous surrogate marker (i.e., the formula should not be used in patients with unstable renal function). At the same SCr, younger patients who have more muscle mass will have a higher GFR than older adults with low muscle mass. Clinical laboratories may begin reporting eGFR based on the CKD-EPI quarrion rather than MDRD equation.[38] The usefulness of the CKD-EPI equation may be particularly evident in younger patients without kidney disease, younger type 1 diabetics without microalbuminuria, or those considering kidney donation with GFR rates approximating normal values. The CKD-EPI equation has replaced the MDRD equation in clinical practice. KDIGO currently recommends the CKD-EPI equation to estimate GFR.[2]

CKD-EPI equation[36,37]:

$$\begin{aligned}\text{GFR (mL/min/1.73 m}^2) &= 141 \times \min\,(S_c/\kappa, 1)^{\alpha} \times \max \\ (S_c/\kappa, 1)^{-1.209} &\times 0.993^{\text{age}} \times 1.018 \text{ (if female)} \times 1.159 \\ &\text{(if African American)}\end{aligned} \tag{12}$$

where S_c = standardized serum creatinine, κ = 0.7 for females and 0.9 for males, α = –0.329 for females and –0.411 for males, min indicates the minimum of S_c/κ or 1, and max indicates the maximum of S_c/κ or 1.

Cystatin C Equations

Recently, there has been calibration and standardization of traceable cystatin C concentrations to International reference standards. The 2012 NKF KDOQI Clinical Practice Guidelines for the Evaluation and Management of CKD recommend measuring cystatin C in adults with eGFR between 45–59 mL/min/1.73 m^2 who do not have confirmatory kidney damage.[2] The measurements of IDMS traceable cystatin C concentrations are not universally available in many community settings.

2012 CKD-EPI cystatin C equation[2]:

$$\begin{aligned}133 &\times \min\,(\text{SCysC}/0.8, 1)^{0.499} \times \max\,(\text{SCysC}/0.8, 1)^{1.328} \\ &\times 0.996^{\text{Age}} \times 0.932 \text{ [if female]}\end{aligned} \tag{13}$$

where SCysC is serum cystatin C (in mg/L), min indicates the minimum of SCysC/0.8 or 1, and max indicates the maximum of SCysC/0.8 or 1.

2012 CKD-EPI creatinine–cystatin C equation[2]:

$$\begin{aligned}135 &\times \min\big(\text{SCr}/k, 1\big)^{\alpha} \times \max\big(\text{SCr}/k, 1\big)^{-0.601} \times \\ \min\big(\text{SCysC}/0.8, 1\big)^{-0.375} &\times \max\big(\text{SCysC}/0.8, 1\big)^{-0.711} \times \\ 0.995^{\text{Age}} &\times \big[0.969 \text{ if female}\big] \times \big[1.08 \text{ if black}\big]\end{aligned} \tag{14}$$

where SCr is serum creatinine (in mg/dL); SCysC is serum cystatin C (in mg/L); k is 0.7 for females and 0.9 for males; a is –0.248 for females and –0.207 for males; min(SCr/k, 1) indicates the minimum of SCr/k or 1, and max(SCr/k, 1) indicates the maximum of SCr/k or 1; and min(SCysC/0.8, 1) indicates the minimum of SCysC/0.8 or 1, and max(SCysC/0.8, 1) indicates the maximum of SCysC/0.8 or 1.

Clinical Controversy: Cockcroft-Gault Versus eGFR Equations

The appropriate dosing of renally eliminated medications is necessary to prevent overdosage or underdosage of medications. Overdosage of a medication can cause significant clinical consequences and contribute to poor patient outcomes. Similarly, underdosing medications can lead to therapeutic failures. In both scenarios, inappropriate medication dosing can lead to increased length of stay, higher healthcare costs, and preventable medication-related problems. The MDRD equation provides a more accurate estimation of kidney excretory function than the Cockcroft-Gault equation.[6,30,39] At this time, the optimal single best equation that can be used universally in all populations does not exist.

The usefulness of the MDRD equation in staging kidney disease is indisputable. Recently, manufacturers have provided some dosage guidance based on eGFR for patients with deteriorating kidney function. Recent studies support the agreement of the MDRD equation with measured GFR and FDA assigned kidney function categories for medication dose adjustment.[40] The NKDEP in 2009 has suggested the use of either the CrCl as estimated by Cockcroft-Gault or the eGFR for dosing medications in CKD for most patients.[41] However, this is controversial as most renal drug dosing is still based on CrCl. An important caveat that is often overlooked in the NKDEP recommendation is that the eGFR needs to be individualized in patients at the extremes in body size by multiplying the eGFR/1.73 m^2 by the patients' body surface area (BSA) to convert units to mL/min:

$$\begin{aligned}\text{Individualized MDRD} &= \text{eGFR/1.73 m}^2 \times \\ \text{estimated BSA (m}^2) &= \text{eGFR for drug dosing}\end{aligned} \tag{15}$$

Alternatively, in patients who are considered to be high risk for adverse medication events, who are taking drugs that have a narrow therapeutic index or where estimations of kidney function vary or are inaccurate, consider measuring CrCl or GFR using exogenous markers.[2] The Nephrology Practice and Research Network of the American College of Clinical Pharmacy has suggested an algorithm for dosing medications eliminated by the kidneys using SCr-based equations.[42] Additionally, safety and efficacy considerations affect decisions regarding dosing of renally eliminated medications that include both patient factors (clinical condition, cachexia) and drug specifics properties (therapeutic index). In summary, individualized patient characteristics and the specific clinical situation necessitate medication dosing decisions based on benefits and risks rather them numbers purely derived from generalized equations.

MEDICATION SAFETY

Pharmacists are responsible for optimizing the use of medications in their patients. Medications eliminated by the kidneys require caution in patients with acute kidney disease and CKD as the need for modifying the drug dose, extending the dosing interval, discontinuing use, and totally avoiding nephrotoxic drugs must be considered. Drug manufacturers provide drug information for use in patients with diminished renal function. Serum levels of medications that are dependent on renal elimination can be elevated contributing to the increased likelihood of drug toxicity and subsequent adverse drug reactions. As mentioned, the use of eGFR estimating equations or direct measurements of CrCl where appropriate can provide important information for medication use and drug dosing. In situations, where SCr is not suitable, consider the use of a cystatin C equation (Equation 14).[2] It cannot be overemphasized—pharmacists' contribution to the care of patients is to ensure appropriate medication use and dosing in patients with acute kidney disease and CKD. In acute kidney disease, temporarily hold administration of drugs that may contribute to or exacerbate kidney damage. **Table 11-3** identifies medication safety considerations for use in patients with acute or CKD. However, specific medication recommendations from more than one reference should be reviewed prior to committing to dosing decisions. (**Minicases 1 and 2**.)

TABLE 11-3. Medication Safety in Patients with CKD[a]

AGENTS	CAUTIONARY NOTES
1. Antihypertensives/cardiac medications	
RAAS antagonists (ACE-Is, ARBs, aldosterone antagonists, direct renin inhibitors)	• Avoid in people with suspected functional renal artery stenosis
	• Start at lower dose in people with GFR <45 mL/min/1.73 m^2
	• Assess GFR and measure serum potassium within 1 wk of starting or following any dose escalation
	• Temporarily suspend during intercurrent illness, planned IV radiocontrast administration, bowel preparation prior to colonoscopy, or prior to major surgery
	• Do not routinely discontinue in people with GFR <30 mL/min/1.73 m^2 as they remain nephroprotective
β-blockers	• Reduce dose by 50% in people with GFR <30 mL/min/1.73 m^2
Digoxin	• Reduce dose based on plasma concentrations
2. Analgesics	
NSAIDs	• Avoid in people with GFR <30 mL/min/1.73 m^2
	• Prolonged therapy is not recommended in people with GFR <60 mL/min/1.73 m^2
	• Should not be used in people taking lithium
	• Avoid in people taking RAAS blocking agents
Opioids	• Reduce dose when GFR <60 mL/min/1.73 m^2
	• Use with caution in people with GFR <15 mL/min/1.73 m^2
3. Antimicrobials	
Penicillin	• Risk of crystalluria when GFR <15 mL/min/1.73 m^2 with high doses
	• Neurotoxicity with benzylpenicillin when GFR <15 mL/min/1.73 m^2 with high doses (maximum 6 g/day)
Aminoglycosides	• Reduce dose and increase dosage interval when GFR <60 mL/min/1.73 m^2
	• Monitor serum levels (trough and peak)
	• Avoid concomitant ototoxic agents such as furosemide
Macrolides	• Reduce dose by 50% when GFR <30 mL/min/1.73 m^2
Fluoroquinolones	• Reduce dose by 50% when GFR <15 mL/min/1.73 m^2
Tetracyclines	• Reduce dose when GFR <45 mL/min/1.73 m^2; can exacerbate uremia
Antifungals	• Avoid amphotericin unless no alternative when GFR <60 mL/min/1.73 m^2
	• Reduce maintenance dose of fluconazole by 50% when GFR <45 mL/min/1.73 m^2
	• Reduce dose of flucytosine when GFR <60 mL/min/1.73 m^2

TABLE 11-3. Medication Safety in Patients with CKD, (cont'd)

AGENTS	CAUTIONARY NOTES
4. Hypoglycemics	
Sulfonylureas	• Avoid agents that are mainly renally excreted (e.g., glyburide/glibenclamide) • Other agents that are mainly metabolized in the liver may need reduced dose when GFR <30 mL/min/1.73 m^2 (e.g., gliclazide, gliquidone)
Insulin	• Partly renally excreted and may need reduced dose when GFR <30 mL/min/1.73 m^2
Metformin	• Suggest avoiding when GFR <30 mL/min/1.73 m^2 , but consider risk-benefit if GFR is stable • Review use when GFR <45 mL/min/1.73 m^2 • Probably safe when GFR ≥45 mL/min/1.73 m^2 • Suspend in people who become acutely unwell
5. Lipid-lowering	
Statins	• No increase in toxicity for simvastatin dosed at 20 mg per day or simvastatin 20 mg/ezetimide 10-mg combinations per day in people with GFR <30 mL/min/1.73 m^2 or on dialysis[83] • Other trials of statins in people with GFR <15 mL/min/1.73 m^2 or on dialysis also showed no excess toxicity
Fenofibrate	• Increases SCr by approximately 0.13 mg/dL (12 μmol/L)
6. Chemotherapeutic	
Cisplatin	• Reduce dose when GFR <60 mL/min/1.73 m^2 • Avoid when GFR <30 mL/min/1.73 m^2
Melphalan	• Reduce dose when GFR <60 mL/min/1.73 m^2
Methotrexate	• Reduce dose when GFR <60 mL/min/1.73 m^2 • Avoid if possible when GFR <15 mL/min/1.73 m^2
7. Anticoagulants	
Low-molecular-weight heparins	• Halve the dose when GFR <30 mL/min/1.73 m^2 • Consider switch to conventional heparin or alternatively monitor plasma anti-factor Xa in those at high risk for bleeding
Warfarin	• Increased risk of bleeding when GFR <30 mL/min/1.73 m^2 • Use lower doses and monitor closely when GFR <30 mL/min/1.73 m^2
8. Miscellaneous	
Lithium	• Nephrotoxic and may cause renal tubular dysfunction with prolonged use even at therapeutic levels • Monitor GFR, electrolytes, and lithium levels monthly or more frequently if the dose changes or the patient is acutely unwell • Avoid using concomitant NSAIDs • Maintain hydration during intercurrent illness • Risk-benefit of drug in specific situation must be weighed

ACE-I = angiotensin-converting enzyme inhibitor; ARB = angiotensin-receptor blocker; CKD = chronic kidney disease; GFR = glomerular filtration rate; IV = intravenous; KDIGO = Kidney Disease: Improving Global Outcomes; NSAIDs = nonsteroidal antiinflammatory drugs; RAAS = renin-angiotensin-aldosterone system; SCr = serum creatinine; wk = week.

[a] Data as of January 2013.

Source: Reproduced with permission from KDIGO.[2]

MINICASE 1

Estimating Equations for GFR

Patricia L., a 47-year-old female (non-African American), has a long standing history for type 1 diabetes mellitus and has been under the care of a nephrologist. Six months ago, her serum creatinine was 1.18 mg/dL.

Laboratory tests today were as follows:

Sodium, 140 mEq/L (136–145 mEq/L)
Potassium, 4.5 mEq/L (3.5–5 mEq/L)
Chloride, 101 mEq/L (96–106 mEq/L)
Carbon dioxide, 28 mEq/L (24–30 mEq/L)
Magnesium, 2 mEq/L (1.5–2.2 mEq/L)
Glucose, 98 mg/dL (70–110 mg/dL)
BUN, 12 mg/dL (8–20 mg/dL)
SCr, 1.49 mg/dL (0.7–1.5 mg/dL)

QUESTION: Which equation should be used to evaluate eGFR and how should these results be interpreted?

DISCUSSION: Using the 2009 CKD-EPI equation (12), the calculated eGFR for this patient was initially 55 mL/min/1.73 m^2 (stage G3 A) and has declined to 41 mL/min/1.73 m^2 (G3 B), respectively. The CKD-EPI equation may permit more effective utilization of resources by better identification of those patients who should be under the care of a nephrologist.[2] The decrease in this patient's GFR needs to be evaluated and further assessed as her kidney disease may be worsening. The consideration of a reversible cause such as volume depletion also warrants further Investigations.

MINICASE 2

Heart Failure

Ruth K., an 83-year-old female with a long history of congestive heart failure, is admitted to Community Hospital with complaints of shortness of breath (she has been sleeping in her recliner and is unable to sleep in her bed despite using two pillows), 15-pound weight gain, and fluid retention in her lower extremities. She also has anorexia, nausea, fatigue, and weakness. All have worsened over the past two weeks.

Physical examination reveals a frail (5'3", 78 kg) woman in moderate distress; HR 108 beats/min; BP 96/60 mm Hg; S3/S4 heart sounds; + 3 pitting edema bilateral lower extremities. Chest x-ray reveals bilateral pleural effusions. PMH: hypertension, osteoarthritis, and atrial fibrillation.

Current medications:
Lisinopril, 20 mg PO daily
Metoprolol succinate, 100 mg PO daily
Furosemide, 40 mg PO daily
KCl, 10 mEq PO BID
Ibuprofen, 400 mg PO four times daily PRN for knee pain

Laboratory tests are as follows:
Sodium, 130 mEq/L (136–142 mEq/L)
Potassium, 3.2 mEq/L (3.8–5 mEq/L)
Chloride, 96 mEq/L (95–103 mEq/L)
Carbon dioxide, 30 mEq/L (24–30 mEq/L or mmol/L)
Magnesium, 1 mEq/L (1.3–2.1 mEq/L)
Glucose, 78 mg/dL (70–110 mg/dL)
Hgb, 11.5 g/dL (12.3–15.3 g/dL)
BUN, 76 mg/dL (8–23 mg/dL)
SCr, 2.5 mg/dL (0.6–1.2 mg/dL)
Urinalysis, normal
BNP, 1200 pg/mL (<100 pg/mL)

Over the next two days, Ruth K. receives aggressive diuretic therapy (furosemide 80 mg IV twice a day), and all electrolyte abnormalities were corrected. Her physical exam was much improved. She was no longer short of breath.

On the morning of day 4, her test results are as follows:

Sodium, 135 mEq/L
Potassium, 3.2 mEq/L
Chloride, 100 mEq/L
Carbon dioxide, 34 mEq/L
Magnesium, 1.4 mEq/L
Glucose, 80 mg/dL
Hgb, 11.4 g/dL
BUN, 35 mg/dL
SCr, 1.4 mg/dL
BNP, 400 pg/mL

QUESTION: What type of renal dysfunction was this patient experiencing on admission to the hospital? What are the likely causes of her elevated BUN and SCr? How often should BUN and SCr be interpreted? Which formula would be best to estimate CrCl or eGFR?

DISCUSSION: This case is rather complex because of the involvement of the kidneys in heart failure. Initially, the elevated BUN and SCr could be attributed to a prerenal state secondary to increased edema (hypervolemia) caused by worsening heart failure. This is supported by her clinical presentation (weight gain, symptoms of heart failure, CXR, elevated BNP, and an elevated BUN:SCr ratio with a ratio of >20:1). The urinalysis does not reveal any cells that might indicate an intrinsic acute kidney injury (see Urinalysis section). In addition, diuretics may increase the BUN, which may complicate the picture, but the other evidence supports the diagnosis of prerenal azotemia. Assessment of kidney function on day 1 is difficult because the Cockcroft-Gault, MDRD, or CKD-EPI equations should not be used in patients with acute alterations in kidney function. In suspected acute kidney injury and when there is a need to assess GFR, measurement of CrCl through collection of urine should be considered.

QUESTION: What has caused this patient's heart failure?

DISCUSSION: She has several risk factors that can worsen heart failure. She has a history of hypertension and atrial fibrillation. She may have been using more ibuprofen more frequently and for an extended period for increased osteoarthritic knee pain. Additional risk factors that could also contribute to exacerbation of heart failure include noncompliance with fluid restriction (2 L) and diet (2 g sodium/day).

QUESTION: What other electrolyte abnormalities have resulted?

DISCUSSION: There are several electrolyte abnormalities identified during initial presentation and then subsequent laboratory analysis: increased BUN and SCr, increased serum bicarbonate, hypokalemia, hypomagnesemia, and hyponatremia. On admission, worsening heart failure resulted in decreased RBF. As with creatinine, there will be a reduction in BUN filtration at the glomerulus; however, urea is avidly reabsorbed in the proximal tubule (following sodium and water) resulting in an elevated ratio of BUN out of proportion to the creatinine (>20:1). This patient also presented initially with hypervolemic hyponatremia. This is most likely caused by worsening heart failure, diminished blood flow to the kidneys, and peripheral edema and subsequent weight gain. As she becomes euvolemic, the hyponatremia will gradually be corrected. After aggressive diuresis with IV furosemide, hypokalemia and hypomagnesemia require replacement therapy. Loop diuretics can also cause metabolic alkalosis (increased serum bicarbonate). Overaggressive diuresis can cause elevations in BUN and SCr without evidence of overt heart failure.

URINALYSIS

Urinalysis is a commonly used clinical tool for the evaluation of various renal and nonrenal problems (e.g., endocrine, metabolic, and genetic). A routine urinalysis is done as a screening test during many hospital admissions and initial physician visits. It is also performed periodically in patients in nursing homes and other settings. The most common components of the urinalysis are discussed here.

An accurate interpretation of a urinalysis can be made only if the urine specimen is properly collected and handled. Techniques are fairly standardized and, keeping in mind that urine is normally sterile, aim to avoid contamination by normal flora of the external environment (mucous membranes of the vagina or uncircumcised penis or by microorganisms on the hands). Therefore, these areas are cleansed and physically kept away from the urine stream. During menses or heavy vaginal secretions, a fresh tampon should be inserted before cleansing.

A first-morning, midstream collection is customarily used as the specimen.[43] Once voided, the urine should be brought to the laboratory as soon as possible to prevent deterioration. If the sample is not refrigerated, bacteria multiply and use glucose (if present) as a food source. Subsequently, glucose concentrations decrease and ketones may evaporate with prolonged standing. Another problem is that formed elements (see Microscopic Analysis section) begin decomposing within two hours. With excessive exposure to light, bilirubin and urobilinogen are oxidized. Unlike other substances, however, protein is minimally affected by prolonged standing.

After the urine sample is collected, it may undergo three types of testing: macroscopic, microscopic, and chemical (dipstick).

Macroscopic Analysis (General Appearance)

The color of normal urine varies greatly—from totally clear to dark yellow or amber—depending on the concentration of solutes. Color comes primarily from the pigments urochrome and urobilin. Fresh normal urine is not cloudy or hazy, but urine may become cloudy if urates (in an acid environment) or phosphates (in an alkaline environment) crystallize or precipitate out of solution. These salts become less soluble as the urine cools from body temperature.

Turbidity may also occur when large numbers of RBCs or white blood cells (WBCs) are present. An unusual amount of foam may be from protein or bile acids. **Table 11-4** lists causes of different urine colors. Some of the changes noted may be urine pH-dependent. In general, drug-induced changes in urine color are fairly rare. Drugs that cause or exacerbate any of the medical problems listed in Table 11-4 can also be considered indirect causes of discolored urine.

Microscopic Analysis (Formed Elements)

Microscopic analysis typically involves the following[44]:

- Centrifuging the urine (12 mL) at 2000 revolutions per minute for five minutes
- Pouring off all "loose" supernatant
- Mixing the sediment with the residual supernatant
- Examining the resulting suspension under 400–440× magnification (also described as high-power field)

TABLE 11-4. Potential Causes of Various Urine Coloring[44-47]

COLOR	CAUSE	POSSIBLE UNDERLYING ETIOLOGIES
Red to orange	Myoglobin	Crush injuries, electric shock, seizures, cocaine-induced muscle damage, rhabdomyolysis
	Hemoglobin/erythrocytes	Hemolysis (malaria, drugs, strenuous exercise), menstrual contamination; kidney stones
	Porphyrins	Porphyria, lead poisoning, liver disease
	Drugs/chemicals	Drugs/chemicals causing above diseases; as dyes: rifampin, isoniazid, riboflavin, sulfasalazine, warfarin, chlorpromazine, thioridazine, phenazopyridine, daunorubicin, doxorubicin, phenolphthalein, phenothiazines, senna, chlorzoxazone
	Food	Beets, rhubarb, blackberries, cold drink dyes, carrots
Blue to green	Biliverdin	Oxidation of bilirubin (poorly preserved specimen)
	Bacteria	*Pseudomonas* or *Proteus* in UTIs (rare), particularly in urine drainage bags
	Drugs/chemicals	As dyes: amitriptyline, azuresin, methylene blue, Clorets abuse, Clinitest ingestion, mitoxantrone, triamterene, resorcinol, promethazine, cimetidine, amitriptyline, metoclopramide, and indomethacin
Brown to black	Myoglobin	Crush injuries, electric shock, seizures, cocaine-induced muscle damage, rhabdomyolysis
	Bile pigments	Hemolysis, bleed into tissues, liver disease
	Melanin	Melanoma (prolonged exposure to air)
	Methemoglobin	Methemoglobinemia from drugs, dyes, etc.
	Porphyrins	Porphyria and sickle cell crisis
	Drugs/chemicals	As dyes: cascara, chloroquine, clofazimine, emodin, senna; as chemicals: ferrous salts, methocarbamol, metronidazole, nitrofurantoin, sulfonamides, sorbitol, α-methyldopa, HMG-CoA reductase Inhibitors, and L-dopa.

HMG-CoA = 3-hydroxy-3-methylglutaryl-coenzyme A; UTIs = urinary tract infections.

Microscopic analysis can be done either routinely or selectively. In either case, one should look for the three "Cs"—cells, casts, and crystals.

Cells

Theoretically, no cells should be seen during microscopic examination of urine. In practice, however, an occasional cell or two is found. These cells include microorganisms, RBCs, WBCs, and tubular epithelial cells.

Microorganisms (normal range: zero to trace). If bacteria are found in the urine sediment, contamination should be the first consideration. Of course, fungi, bacteria, and other single-cell organisms can be seen in patients with a urinary tract infection (UTI) or colonization. Even if ordered, some laboratories do not perform urine cultures unless there is significant bacteriuria. *Significant bacteriuria* may be defined as an initial positive dipstick screen for leukocyte esterase and nitrites (Chemical Analysis section). Likewise, some laboratories do not process cultures further (e.g., identification, quantification, and susceptibility) if more than one or two different bacterial species is seen on initial plating. Additionally, some laboratories do not perform susceptibility testing if more than one organism (some more than two) is isolated or if <100,000 (some use 50,000 as the cutoff) colony-forming units (CFU) per milliliter per organism are measured with a midstream, clean-catch sample. The common cutoff for urine obtained through a catheter is <10,000 CFU/mL/organism. If multiple types of bacteria are present, contamination by flora from vaginal, rectal, hand, skin, or other body sites is assumed.

Red blood cells (erythrocytes) (normal range: one to three per high-power field). Hematuria is the abnormal renal excretion of erythrocytes detected in two of three urine samples. A few RBCs are occasionally found in the urine of a healthy man or woman, particularly after exertion, trauma, or fever. If persistent, even small numbers (greater than two to three per high-powered field) may reflect urinary tract pathology. Increased numbers of RBCs are seen (among others) in glomerulonephritis, infection (pyelonephritis), renal infarction or papillary necrosis, tumors, stones, and coagulopathies. In some of these disorders, hematuria may turn the urine pink or red (gross hematuria). If the specimen is not collected properly, vaginal blood may contaminate the urine. Many squamous epithelial cells also appear in this case, suggesting that the erythrocytes did not originate from the urinary tract, but probably from the vaginal walls.[46,48,49]

White blood cells (leukocytes) (normal range: zero to two per high-power field). Potentially significant pyuria has been defined as three or more WBCs per high-power field of centrifuged urine sediment. Pyuria is usually associated with UTIs (upper or lower). However, inflammatory conditions (glomerulonephritis, interstitial nephritis) may also lead to this finding.[44,46]

Tubular epithelial cells (normal range: zero or one per high-power field). One epithelial cell per high-power field is often found in normal subjects. Cells originating from the renal tubules are small, oval, and mononuclear. Their quantity increases dramatically when the tubules are damaged (e.g., acute tubular necrosis) or when there is inflammation from interstitial nephritis or glomerulonephritis.[44]

Casts

Casts are cylindrical masses of glycoproteins (e.g., Tamm-Horsfall mucoprotein) that form in the tubules. Casts have relatively smooth and regular margins (as opposed to clumps of cells) because they conform to the shape of the tubular lumen. Under certain conditions, casts are released into the urine (called *cylindruria*). Even normal urine can contain a few clear casts. These formed elements are fragile and dissolve more quickly in warm, alkaline urine. Types include hyaline, cellular, granular, and waxy (broad); their causes are listed in **Table 11-5.**

Hyaline casts. Being clear, hyaline casts are difficult to observe under a microscope and are, by themselves, not indicative of disease. Hyaline casts can be seen in concentrated urine or with the use of diuretics.[46,49]

Cellular casts. In contrast to hyaline casts, cellular casts are seen with intrinsic renal disease. They form when leukocytes, RBCs, or renal tubular epithelial cells become entrapped in the gelatinous matrix forming in the tubule. Their clinical significance is the same as that of the cells themselves; unlike free cells, however, cells in casts originate from within the kidneys. The identification of a particular cast-type is often used to assist in diagnosis. WBC casts suggest intrarenal inflammation (e.g., acute interstitial nephritis) or pyelonephritis. Epithelial cell casts suggest tubular destruction; they may also be noted in glomerulonephritis. RBC casts are seen in glomerulonephritis.[44,49]

Granular and waxy casts. Granular and waxy casts are older, degenerated forms of the other types. Granular (also called *muddy brown*) casts can be seen in many conditions such as acute tubular necrosis, glomerulonephritis, and tubulointerstitial disease. Because waxy casts occur in many diseases, they do not offer much diagnostic information.[44,49]

TABLE 11-5. Causes of Various Types of Casts in Urine[44,46,48]

CAST	CAUSE
Red blood cell	Classically seen with acute glomerulonephritis; can be seen in patients who play contact sports and uncommonly with tubular interstitial disease
White blood cell	Classically seen with pyelonephritis; also seen with glomerulonephritis and interstitial nephritis
Tubular epithelial cell	Nonspecific; acute tubular necrosis, glomerulonephritis, tubulointerstitial disease; also seen with cytomegalovirus infection and toxicity from salicylates and heavy metals, ethylene glycol
Hyaline casts	Nonspecific and may not be pathologic; seen with prerenal azotemia and strenuous exercise
Granular casts	Nonspecific but pathologic; may be seen in acute tubular necrosis; volume depletion, glomerulonephritis, tubulointerstitial disease
Waxy (broad) casts	Nonspecific but pathologic; may be seen with advanced or chronic renal failure

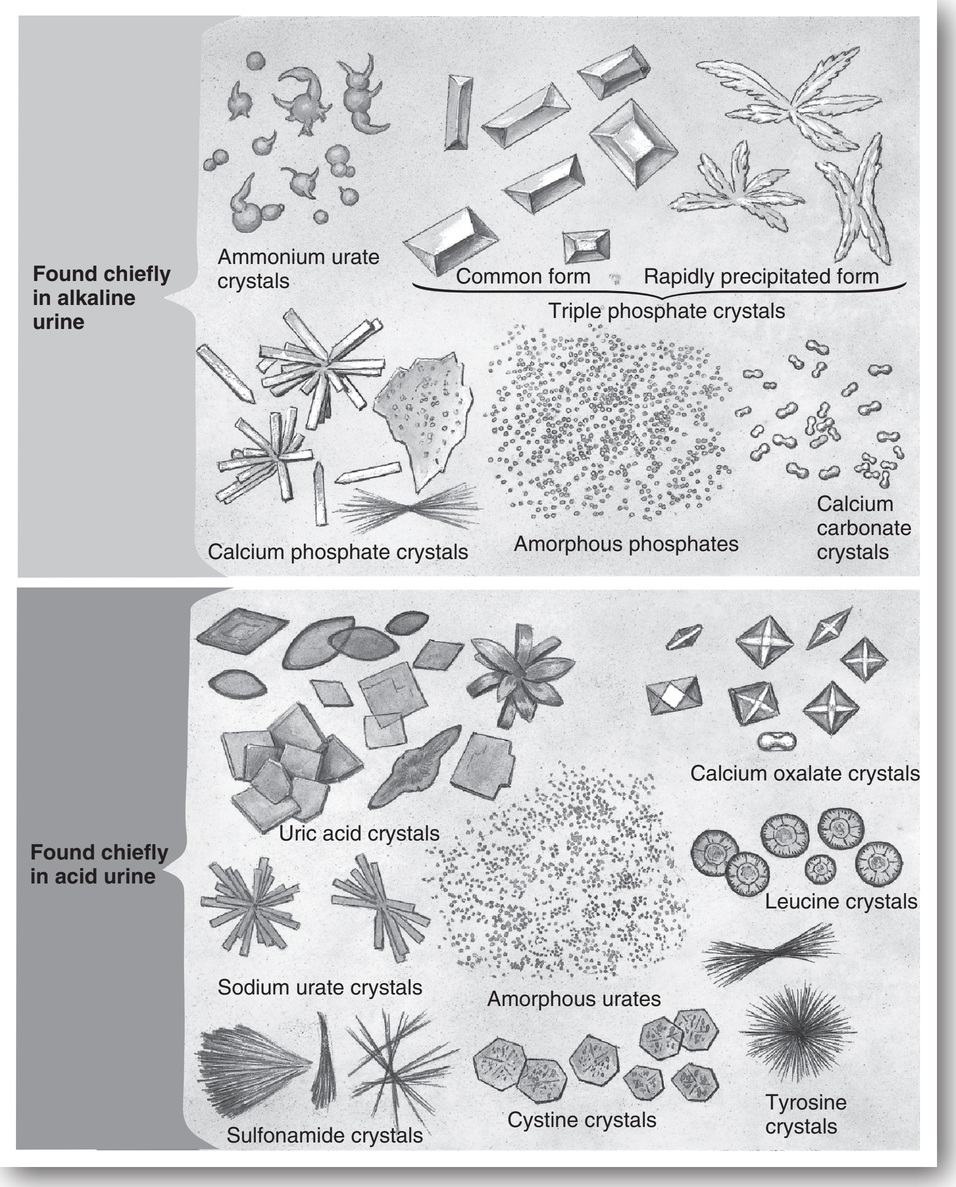
Found chiefly in alkaline urine
Ammonium urate crystals
Common form
Rapidly precipitated form
Triple phosphate crystals
Calcium phosphate crystals
Amorphous phosphates
Calcium carbonate crystals
Found chiefly in acid urine
Uric acid crystals
Calcium oxalate crystals
Leucine crystals
Sodium urate crystals
Amorphous urates
Sulfonamide crystals
Cystine crystals
Tyrosine crystals

FIGURE 11-3. Inorganic elements that may be found in urinary sediment. Elements founds in alkaline urine are represented in the top figure and those found in alkaline urine are represented on the bottom. *Source*: Reprinted with permission from Runge MS, Greganti MA. Netter's internal medicine. 2nd ed. Philadelphia: Saunders; 2008:766. Copyright© 2008. Netter medical illustration used with permission of Elsevier. All rights reserved.

Crystals

The presence of *crystals* in the urine depends on urinary pH, the degree of saturation of the urine by the substance that is forming crystals, and the presence of other substances in the urine that may promote crystallization. There are numerous types of crystals that can be detected in the urine (Figure 11-3). Crystalluria, if differentiated by type, can help to identify patients with certain local and systemic diseases. Cystine crystals occur with the condition cystinuria, and struvite (magnesium ammonium phosphate) crystals are seen with struvite stones. Calcium oxalate, calcium phosphate, and uric acid crystals are also suggestive of stones. Many crystals can be detected in otherwise healthy patients.[45,49]

CHEMICAL ANALYSIS (SEMIQUANTITATIVE TESTS, URINE DIPSTICK TESTS)

For this discussion, biochemical analysis of urine includes protein; pH; specific gravity; bilirubin, bile, and urobilinogen; blood and hemoglobin; leukocyte esterase; nitrite; glucose; and ketones. All of these semiquantitative tests can be performed quickly using modern dipsticks containing one or more reagent-impregnated pads. When using these strips, the clinician must carefully apply the urine to the pads as instructed and wait the designated time before comparing pad colors to the color chart. Possible results associated with various colors are displayed in **Table 11-6.**

TABLE 11-6. Examples of Tests Available and Possible Results from Multitest Urine Dipstick (Bayer Multistix 10 SG)

TEST	RESULT						
Leukocyte esterase	Negative	Trace	Small +	Moderate ++	Large +++		
Nitrite	Negative	Positive					
Urobilinogen	Normal 0.2 mg/dL	Normal 1 mg/dL	2 mg/dL	4 mg/dL	8 mg/dL		
Protein	Negative	Trace	30 mg/dL +	100 mg/dL ++	300 mg/dL +++	2000 mg/dL ++++	
pH	5	6	6.5	7	7.5	8	8.5
Blood (Hgb)	Negative	Nonhemolyzed Trace	Hemolyzed Trace	Small +	Moderate ++	Large +++	
Specific gravity	1.000	1.005	1.010	1.015	1.020	1.025	1.030
Ketones	Negative	Trace 5 mg/dL	Small 15 mg/dL	Moderate 40 mg/dL	Large 80 mg/dL	Large 160 mg/dL	
Bilirubin	Negative	Small +	Moderate ++	Large +++			
Glucose	Negative	1/10 g/dL (trace) 100 mg/dL	1/4 g/dL 250 mg/dL	1/2 g/dL 500 mg/dL	1 g/dL 1000 mg/dL	2 g/dL 2000 mg/dL	

Protein

Normal range: zero to trace on dipstick or <200 mg/g (urine protein to creatinine ratio)

The normal urinary proteins are albumin and low molecular weight serum globulins. The glomerulus is freely permeable to substances with a molecular weight of <20,000 daltons. However, albumin with a molecular weight of 65,000 daltons is typically restricted from passing through the glomerulus into the urine. The smaller serum globulins that are filtered in the nephron are generally reabsorbed in the proximal tubule. Therefore, healthy individuals excrete very small amounts of protein in the urine (about 80–100 mg of protein per day). In the presence of kidney damage, larger quantities of protein may be excreted. Increased excretion of albumin is associated with diabetic nephropathy, glomerular disease, and uncontrolled hypertension. If low molecular globulins are detected, it is more likely a tubulointerstitial process. The term *proteinuria* is a general term that refers to the renal loss of protein (albumin and globulins). The term *albuminuria* specifically refers to the abnormal renal excretion of albumin. Clinical proteinuria is defined as the loss of >500 mg/day of protein urine. Patients with microalbuminuria are excreting relatively small, but still pathogenic, amounts (30–300 mg/day) of albumin. Common causes of proteinuria are listed in **Table 11-7**. It should be noted that proteinuria is sometimes intermittent and is not always pathologic (e.g., after exercise and fever).

Because of difficulties with overnight and 24-hour collections, KDIGO recommends spot (untimed) urine testing. The ACR ratio is convenient and accounts for urine volume effects on protein concentration and standardizes the protein or albumin excretion to creatinine excretion. The ratio of protein (or albumin) to creatinine in an untimed urine sample is a very accurate estimate of the total amount of protein (or albumin) excreted in the urine over 24 hours.[2] The current criteria for staging CKD and prognosis of CKD recommends the measurement to detect albuminuria. The KDIGO working group recommends the urine ACR ratio as the preferred method to assess for kidney damage in addition to estimation of GFR.[2]

Color indicator test strips (e.g., Albustix, Multistix) used to detect and measure protein in the urine contain a buffer mixed with a dye (usually tetrabromophenol blue). In the absence of albumin, the buffer holds the pH at 3, maintaining a yellow color. If albumin is present, it reduces the activity coefficient of hydrogen ions (the pH rises), producing a blue

TABLE 11-7. Causes of Proteins in Urine[43]

Mild proteinuria (<0.5 g/day)
- High blood pressure
- Lower UTI
- Fever
- Renal tubular damage
- Exercise

Moderate proteinuria (0.5–3 g/day)
- Congestive heart failure
- Chronic glomerulonephritis
- Acute glomerulonephritis
- Diabetic nephropathy
- Pyelonephritis
- Multiple myeloma
- Preeclampsia of pregnancy

Significant proteinuria (>3 g/day)
- Glomerulonephritis
- Amyloid
- Chronic glomerulonephritis (severe)
- Diabetic nephropathy
- Lupus nephritis

UTI = urinary tract infection.

color. Of note, these tests are fairly insensitive to the presence of low molecular globulins including the Bence-Jones protein. Results can be affected by the urinary concentration. At both extremes of urinary concentrations, false positives and false negatives may occur. The potential for this can be easily assessed if specific gravity is measured concomitantly. Substances that cause abnormal urine color may affect the readability of the strips. These include blood, bilirubin, phenazopyridine nitrofurantoin, and riboflavin.[50] Standard dipsticks do not detect microalbuminuria; however, newer dye-impregnated strips are available that can detect lower concentrations of albumin

pH

Normal range: 4.6–8

Sulfuric acid, resulting from the metabolism of sulfur-containing amino acids, is the primary acid generated by the daily ingestion of food. The pH is usually estimated in 0.5-unit increments by use of test strips containing methyl red and bromthymol blue indicators. These strips undergo a series of color changes from orange to blue over a pH range of 5–8.5. In addition, pH can be precisely measured with electronic pH meters. Normally, the kidneys can eliminate the acid load by excreting acid itself and sodium hydroxide ions. In fact, healthy persons can acidify the urine to pH 4.5, although the average pH is around 6. Any pH close to the reference range can be interpreted as normal as long as it reflects the kidneys' attempts at regulating blood pH. The urinary pH can be affected by the various acid–base disorders. Determination of the urinary pH is often used in the setting of a UTI.[48,49] (**Minicase 3**.) In general, acidic (versus neutral) urine deters bacterial colonization. Alkaline urine may be seen with either UTIs caused by urea-splitting bacteria, such as *Proteus mirabilis* (via ammonia production), or tubular defects causing decreased net tubular hydrogen ion secretion as in renal tubular acidosis.

By their intended or unintended pharmacological actions, drugs also can cause true pH changes; they do not interfere

MINICASE 3

Urinary Tract Infection

Elizabeth R. is a 75-year-old woman residing at a long-term care facility while rehabilitating from a total hip replacement. She has a past medical history of hypertension, osteoarthritis, hypothyroidism, hyperlipidemia, osteoporosis, and gastroesophageal reflux disease. Today, she is confused, feels fatigued, and reports frequent voiding, dysuria, and suprapubic tenderness. Her temperature is 101.8 °F (38.8 °C). Urine culture reveals >100,000 CFU/mL *Escherichia coli*, which is sensitive to all antibiotics except ampicillin. Laboratory results from two weeks ago demonstrate an eGFR of 75 mL/min. The results of her urine analysis are listed below:

URINE ANALYSIS	RESULT	REFERENCE STANDARD/RANGES
Color	Yellow, cloudy	Yellow
Specific gravity	1.019	1.016–1.022
pH	7	4.6–8
Blood	1+	Negative
Protein	Negative	Negative
Nitrite	1+	Negative
Leukocyte esterase	3+	Negative-trace
Bacteria	3+	0–trace
WBCs	60–100/HPF	0–2/HPF
RBCs	2/HPF	1–3/HPF
Epithelial cells	0	0–1/HPF

QUESTION: Does this patient have a UTI? What elements of the urine analysis are important?

DISCUSSION: In this older woman, the urine analysis alone cannot determine whether she has a UTI. The clinical correlation requires positive symptoms such as fever, acute dysuria, frequent or worsening urinary frequency, suprapubic pain, and costovertebral angle tenderness or pain. She does have four positive symptoms. Reviewing the urine analysis, the abnormal results indicative of an infection are the presence of the positive nitrite, leukocyte esterase, and bacteria. Asymptomatic pyuria is a frequent and relevant finding in nursing home patients and needs to be correlated with the clinical presentation. The urine culture identifying *Escherichia coli* >100,000 CFU and sensitivity report can be very useful in selecting the best therapeutic medication. Further information like patient allergies and an eGFR can help the clinician select the most appropriate antimicrobial agent.

TABLE 11-8. Factors Affecting Urine pH[43,46]

URINE PH AND FACTORS	CAUSES AND COMMENTS
Alkaline urine	
Postprandial	Specimens voided shortly after meals
Vegetarianism	Vegetables do not produce fixed acid residues
Alkalosis (metabolic or respiratory)	Hyperventilation, severe vomiting, GI suctioning
UTI	Some bacteria (e.g., *Proteus*) split urea to ammonia, which is alkalinizing
Renal tubular acidosis	Impaired tubular acidification of urine and low bicarbonate and pH in blood
Drugs	Acetazolamide, bicarbonate salts, thiazides, citrate, and acetate salts
Acidic urine	
Drugs	Ammonium chloride, ascorbic acid (high dose), methenamine
Food	Cranberries, prunes, plums, fruit juices
Ketoacidosis	Diabetes mellitus, starvation, high fever
Metabolic acidosis	Increased ammonium excretion and cellular hypoxia with lactic acid production (shock)
Sleep	Mild respiratory acidosis

GI = gastrointestinal; UTI = urinary tract infection.

with the reagents used to estimate urine pH. Drugs that induce diseases associated with pH changes are indirect causes. These and other causes of acidic and alkaline urine are listed in **Table 11-8.** Persistent pHs >7 are associated with calcium carbonate, calcium phosphate, and magnesium–ammonium phosphate stones; pHs <5.5 are associated with cystine and uric acid stones.

Specific Gravity

Normal range: 1.016–1.022 (normal fluid intake)

The kidneys are responsible for maintaining the blood's osmolality within a narrow range (285–300 mOsm/kg). To do so, the kidneys must vary the osmolality of the urine over a wide range. Although osmolality is the best measure of the kidneys' concentrating ability, determining osmolality is difficult. Fortunately, it correlates well with specific gravity when the urine contains normal constituents. *Specific gravity* is the ratio of the weight of a given fluid to the weight of an equal volume of distilled water. Sodium, urea, sulfate, and phosphate contribute most to the specific gravity of urine. Because specific gravity is related to the weight (and not the number) of particles in solution, particles with a weight different from that of sodium chloride (the solute usually in the highest concentration there) can widen the disparity. Patients with normal kidney function can dilute urine to approximately 1.001 and concentrate urine to 1.035, which correlates to an osmolality of 50–1000 mOsm/kg, respectively. A urinary specific gravity of 1.010 is considered isosthenuric; that is, the urinary osmolality is the same as plasma.[44,46,48]

Specific gravity can be measured by reagent strips (dipstick), a urinometer (hydrometer), or a refractometer. The reagent strips change color based on the pK_a change of the strips in relation to the ionic concentration of the urine. The indicator substance on the strip changes color, which can be then correlated to the specific gravity. Specific gravity measured by reagent strips is not affected by high concentrations of substances like glucose, protein, or radiographic contrast media, which may elevate readings with refractometers and urinometers. The urinometer is akin to a graduated buoy; it requires sufficient urine volume to float freely. The reading is adjusted according to the urine temperature. The refractometer uses the refractive index as a basis and needs only a few milliliters of urine and no temperature adjustment.[44,46,48]

Several conditions can affect specific gravity. In general, urinary specific gravity should be considered abnormal if it is the opposite (high verus low and vice versa) of that which should be produced based on the concurrent plasma osmolality. Patients who are volume depleted should present with a concentrated urine (specific gravity ≥1.020) as a normal compensatory mechanism. Patients with prerenal disease will likely have relatively concentrated urine, while those with intrinsic damage to the renal tubules are more likely to produce urine, which is isosthenuric (the tubules are unable to dilute or concentrate the urine so the urine is the same concentration as the filtrate). The urine of patients with diabetes insipidus has low values (<1.005) despite a relatively hypertonic plasma. On the other hand, patients with the syndrome of inappropriate secretion of antidiuretic hormone (SIADH) have concentrated urine and relatively hypotonic serum.[44,46,48]

Urobilinogen

Normal range: 0.3–1 Ehrlich unit

Urobilinogen (formed by bacterial conversion of conjugated bilirubin in the intestine) is normally present in urine and increases when the turnover of heme pigments is abnormally rapid, as in hemolytic anemia, congestive heart failure with liver congestion, cirrhosis, viral hepatitis, and drug-induced hepatotoxicity. Elevated urobilinogen may be premonitory of early hepatocellular injury, such as hepatitis, because it is evident in urine before serum bilirubin levels increase. Alkaline urine is also associated with increased urobilinogen concentrations due to enhanced renal elimination. Urobilinogen may decrease (if previously elevated) in patients started on antibiotics (e.g., neomycin, chloramphenicol, and tetracycline) that reduce the intestinal flora producing this substance. Urobilinogen is usually absent in total biliary obstruction, because the substance cannot be formed. Increased urobilinogen in the absence of bilirubin in the urine suggests a hemolytic process.

Bilirubin

Normal range: negative

A dark yellow or greenish-brown color generally suggests *bilirubin* in the urine (bilirubinuria). Most test strips rely on the reaction between bilirubin with a diazotized organic dye to yield a distinct color. Bilirubinuria may be seen in patients with intrahepatic cholestasis or obstruction of the bile duct (stones or tumor). False-negative results may occur in patients taking ascorbic acid.

Blood and Hemoglobin

Normal range: negative

Dipsticks for blood are dependent on the oxidation of an indicator dye due to the peroxidase activity of hemoglobin. A dipstick test can detect as few as one to two RBCs per high-power field. Even small amounts of blood noted on dipstick require further investigation. It is important to note that in addition to hemoglobin, myoglobin can also catalyze this reaction so that a positive dipstick for blood may indicate hematuria (blood), hemoglobinuria (free hemoglobin in urine), or myoglobinuria. Microscopic examination of the urine is needed to distinguish hematuria. The presence of ascorbic acid in the urine may lead to a false negative with these tests; this is usually associated with a fairly large oral intake of vitamin C.[46,48, 51]

Hemoglobinuria suggests the presence in intravascular hemolysis or directed damage to the small blood vessels. The presence of myoglobin in the urine is highly suggestive of rhabdomyolysis, the acute destruction of muscle cells. With rhabdomyolysis, myoglobin is cleared rapidly by the kidneys and can be detected in the urine.[46]

The clinical distinction between hematuria, hemoglobinuria, and myoglobinuria is important because the clinical conditions that cause them are very different. The color of the urine is not specific; all three may lead to red or dark brown urine. As noted, with dipsticks for blood, all three conditions will lead to a positive test. Microscopic analysis will demonstrate many more erythrocytes with hematuria, but RBCs can be seen with hemoglobinuria and myoglobinuria. Erythrocytes may be few in number in hematuria due to lysis of the RBCs if the urine has a low specific gravity (<1.005).

Leukocyte Esterase

Normal range: negative to trace

Many dipsticks can detect *leukocyte esterase,* give a semiquantitative estimate of pyuria (pus in the urine), and, thus, be considered an indirect test for UTIs. The presence of esterase activity correlates well with significant numbers of neutrophils (either present or lysed) in the urine. The leukocyte esterase test is important because the presence of actual neutrophils in the urine is not a specific indicator for UTI.[46,48]

Nitrite

Normal range: negative

The presence of *nitrite* in the urine is another indirect indicator of a UTI. Many organisms such as *Escherichia coli, Klebsiella, Enterobacter, Proteus, Staphylococcus,* and *Pseudomonas* are able to reduce nitrate to nitrite, and, thus, a positive urine test would suggest a UTI. If nitrite-positive, a culture of the urine should be obtained. A first-morning urine specimen is preferred because an incubation period is necessary for the bacteria to convert urinary nitrate to nitrite. A positive test is suggestive of a UTI, but a negative test cannot rule out a UTI (i.e., the test is specific but not highly sensitive). False-positive tests may be due to strips that are exposed to air. False negatives occur with infections caused by non-nitrite-producing organisms (*Enterococcus*).[46,48]

Glucose and Ketones

Normal range: none

Although glucose is filtered in the glomerulus, it is almost completely reabsorbed in the proximal tubule so that glucose is generally absent in the urine. However, at glucose concentrations >180 mg/dL, the capacity to reabsorb glucose is exceeded and glycosuria will occur. Glucose in the urine is suggestive of diabetes mellitus although other, less common conditions can cause glycosuria. The use of urinary glucose to screen and monitor for diabetes is no longer a standard of care.[46,48,49]

Ketones in the urine typically indicate a derangement of carbohydrate metabolism resulting in utilization of fatty acids as an energy source. Ketonuria in association with glucose in the urine is suggestive of uncontrolled type 1 diabetes mellitus. Ketonuria can also occur with pregnancy, carbohydrate-free diets, and starvation. Aspirin has been reported to cause a false-negative ketone test, whereas levodopa and phenazopyridine may cause false-positive ketone results.[46,48,49]

Urinary Electrolytes

Like most laboratory tests, *urinary electrolytes* are rarely definitive for any diagnosis. They can confirm suspicions of a particular medical problem from the history, physical examination, and other laboratory data. Along with the results of a urinalysis and serum electrolytes, urinary electrolyte tests allow the practitioner to rule in or out possible diseases of the differential diagnosis. These tests are relatively simple to perform and widely used in the clinical setting.

“Normal” values for urinary electrolytes are a bit of a misnomer because the kidneys should be retaining or excreting electrolytes based on intake and any endogenous production. So any concentration in the urine is normal if it favors a normal fluid and serum electrolyte status. A related test, the urinary fractional excretion of sodium ($\%FE_{Na}$), can assist with common diagnostic dilemmas involving the kidneys’ ability to regulate electrolytes.

Urinary Sodium and Potassium

The electrolyte that is most commonly measured in the urine is sodium. Occasionally, it is also useful to measure potassium and chloride. For these electrolytes, there is no conversion factor to SI (International System) units because milliequivalents per liter are equivalent to millimoles per liter.

Sodium

Normal range: varies widely

Regulation of urinary excretion of sodium maintains an effective systemic circulating volume. For this reason, the urinary sodium concentration is often used to assess volume status in a patient. Less often, a 24-hour assessment of sodium excretion (via a urine collection) can be used to assess adherence to sodium restriction in a patient with hypertension and heart failure.[52,53] This is because the total urinary sodium excretion should equal the amount of sodium taken in through the diet. For example a patient following a low sodium diet should ingest <90 mEq (90 mmol) of sodium per day and would, therefore, have a 24-hour urine sodium <90 mEq (90 mmol) per day if they are following the diet accurately. Sodium and water balance is an extremely complex process, and only the most common disorders that may alter sodium and water balance (and hence urine sodium) are discussed here.

Hyponatremia is the most common electrolyte disorder seen in clinical practice, and it is most often observed in volume depletion (GI loss and diuretics) and in SIADH, which is not uncommon. In particular, SIADH can be seen in the elderly who are maintained on drugs known to cause excess secretion of ADH, such as the selective serotonin reuptake inhibitors. Urine sodium concentrations of <20 mEq/L generally suggest volume depletion—the kidneys are responding to the low volume by reabsorbing sodium. In the case of SIADH, which is characterized by inappropriate retention of water in the distal tubule, the urine sodium is generally >20–40 mEq/L.

Hypernatremia is less common and occurs when there is limited access to free water because otherwise healthy adults will become thirsty in the face of hypernatremia. Diabetes insipidus, which is characterized by a decreased production or response to ADH, is another cause of hypernatremia. With diabetes insipidus, the urine sodium concentration will be low despite the presence of clinical euvolemia. This is due to dilution of the urinary sodium secondary to inappropriate loss of water in the urine.[52-54]

Urine sodium concentrations are also useful in the diagnosis of acute kidney injury. In the presence of prerenal azotemia, urine sodium concentrations are low. This is due to the kidneys' attempt to maintain volume and blood flow to the kidneys. On the other hand, with acute tubular necrosis, the urinary sodium is generally >40 mEq/L because the damaged renal tubules are unable to reabsorb sodium and concentrate urine.[52,53] Diuretics can interfere with the assessment of urinary sodium. Even with volume depletion, the urinary sodium can be high due to the effect of the diuretic on renal sodium handling.[52,53]

Potassium

Normal range: varies widely

As is the case with sodium, the urinary excretion of potassium varies based on dietary intake and other factors that may affect serum potassium concentrations. For patients with unexplained hypokalemia, urinary potassium may provide useful information. Concentrations >10 mEq/L in a hypokalemic patient usually mean that the kidneys are responsible for the loss. This may occur with potassium-wasting diuretics, high-dose sodium penicillin therapy (e.g., ticarcillin/clavulanate and piperacillin/tazobactam), metabolic acidosis or alkalosis, and renal tubular acidosis. Concomitant hypokalemia and low urinary potassium (<10 mEq/L) suggest GI loss (including chronic laxative abuse) as the cause of low serum potassium. In the setting of hyperkalemia, assessment of urinary potassium concentrations is less useful. Hyperkalemia is often due to kidney failure (with or without drugs that affect potassium homeostasis) so potassium concentrations in the urine would be low.[52,53]

%FE_{Na} Test

Although assessment of urine sodium concentrations is very useful in determining volume status, the concentration of sodium in the urine is affected by the degree of water reabsorption in the tubules. The FE_{Na} is the % of sodium (fraction) that is filtered in the glomerulus that eventually is excreted in the urine and, thus, corrects for the amount of water in the filtrate. An FE_{Na} can be estimated from a spot (random) urine sample with a concomitant serum sample. The calculation is

$$FE_{Na}(\%) = \frac{U_{Na} \times S_{cr}}{S_{Na} \times U_{cr}} \times 100 \tag{16}$$

where U_{Na} and S_{Na} are urine and serum sodium in milliequivalents per liter or millimoles per liter, and U_{Cr} and S_{Cr} are in milligrams per deciliter or micromoles per liter.

In the face of acute kidney injury, the FE_{Na} can be useful to discriminate between a prerenal process (i.e., volume depletion) and acute tubular necrosis. In the hypovolemic, prerenal state, the kidneys will conserve sodium and the FE_{Na} will be <1%. With tubular damage, the FE_{Na} will generally be >2–3%. As with the assessment of urine sodium, the FE_{Na} can be affected by diuretic therapy and may be somewhat high despite volume depletion.[52,53]

SUMMARY

The kidneys play a major role in the regulation of fluids, electrolytes, and the acid–base balance. Kidney function is affected by the cardiovascular, pulmonary, endocrine, and central nervous systems. Therefore, abnormalities in these systems may be reflected in renal or urine tests. The urinalysis is useful as a mirror for organ systems that generate substances (e.g., blood/biliary system and urobilinogen) ultimately eliminated in the urine. A urinalysis allows indirect examination without invasive procedures.

A rise in BUN without a simultaneous rise in SCr is not specific for kidney dysfunction. However, concomitant elevations in BUN and SCr almost always reflect some disturbance in the kidneys' ability to clear substances from the body. Renal functions should be estimated based on the patient's SCr and demographic characteristics using either the 2009 CKD-EPI or Cockcroft-Gault equations. These equations are a more reliable index of kidney function than SCr alone. Evolving evidence may show better estimation of GFR with creatinine–cystatin-C equations.[55] A thoughtful examination of the urine (macroscopic, microscopic, and chemical) is an indispensable tool in identifying kidney and other pathological processes that may be present in a patient.

LEARNING POINTS

1. ***What is the relevance in knowing the eGFR?***

   ANSWER: The importance is in the assessment of whether the GFR is stable or changing. Classification of kidney disease is based on estimates of GFR and ranges of albuminuria, and identifies those patients who need to be under the care of nephrologists. Also, assessing for changes in GFR may be reflective of worsening disease severity. There are many medications eliminated by renal excretion. Inappropriate use of nephrotoxic drugs or inappropriate dosing in patients with reduced renal function as evidenced by low eGFR may contribute to adverse drug reactions. The eGFR may assist the pharmacist in the assessment of medication use and the determination of dose and frequency adjustment.

2. ***Which is better to use for drug dosing—the Cockcroft-Gault, MDRD, or CKD-EPI equation?***

   ANSWER: Either the CrCl using CG or the eGFR multiplied by BSA may be used to calculate drug doses for most patients. Consider measuring CrCl for patients that are considered to be high risk (youngest and very old), for patients receiving drugs that have a narrow therapeutic index, or for patients in whom estimations of kidney function vary or are likely to be inaccurate.

3. ***What is the clinical relevance of a urinary fractional excretion of sodium (%FE_{Na})?***

   ANSWER: The FE_{Na} reflects the percent of filtered sodium that is ultimately excreted in the urine. Low FE_{Na} values indicate that the kidneys are attempting to conserve sodium and water; thus, the patient is in a prerenal state. Other indicators of prerenal kidney dysfunction include a low urinary sodium value and a high urine osmolality (indicating a concentrated urine). A high FE_{Na} is less specific. This may occur in a well-hydrated patient, a patient with acute tubular necrosis, or in a patient on diuretics.

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QUICKVIEW | BUN

PARAMETER	DESCRIPTION	COMMENTS
Common reference range		
Adults	8–23 mg/dL (2.9–8.2 mmol/L)	BUN represents the concentration of nitrogen in the serum Usually measured with creatinine to assess renal function A normal BUN:creatinine ratio is 6:1 to 20:1; if the ratio is >20:1, it suggests a prerenal etiology of renal failure; if the ratio is 10–20:1, it suggests an intrarenal etiology of renal failure
Pediatrics	5–18 mg/dL (1.8–6.4 mmol/L)	
Critical value	100 mg/dL (35.7 mmol/L)	Associated with uremic syndrome in patients with severe renal failure
Inherent activity	Extremely high BUN levels lead to uremia, which includes symptoms of nausea, vomiting, and other metabolic and endocrine abnormalities	
Location		
Production	Urea is a byproduct of hepatic protein metabolism; the source of protein can be exogenous (e.g., protein in the diet) or endogenous (e.g., breakdown of RBCs or muscle cells)	Urea is the primary way that the body eliminates excess nitrogen from the body
Storage	Not applicable	
Secretion/excretion	Urea is 100% filtered by the glomerulus and then undergoes proximal tubule reabsorption	The percentage that is reabsorbed by the proximal tubule is inversely related to the patient's intravascular volume; if the intravascular volume is lower than normal, then the percentage of BUN reabsorbed in the proximal tubule is increased
Causes of abnormal values		
High	Prerenal causes: dehydration, blood loss, shock, congestive heart failure, hypotension, increased protein catabolism (due to fever, infection, severe burns, etc.) Intrarenal causes: acute or chronic renal failure due to any cause, glomerulonephritis, acute tubular necrosis, severe hypertension Postrenal causes: obstruction of ureter, bladder neck, or urethra due to stones, enlarged prostate, or stricture, respectively Nonrenal causes: excessive amino acid infusions, upper gastrointestinal tract bleeding Drugs with antianabolic effects or protein catabolic effects: corticosteroids, tetracyclines Drugs that contribute to prerenal or intrarenal failure: angiotensin-converting enzyme inhibitor, acetaminophen, acyclovir, diuretics, aminoglycosides, antibiotics, angiotensin II receptor blockers, nonsteroidal anti-inflammatory agents, radiographic contract media	
Low	Starving or malnourished patients with inadequate protein intake or patients with muscle wasting disease Excess intravascular volume (e.g., congestive heart failure or SIADH may dilute BUN and have very low levels Chloramphenicol, guanethidine, or streptomycin use	

Continued

QUICKVIEW | BUN (cont'd)

PARAMETER	DESCRIPTION	COMMENTS
Signs and symptoms		
High level	Azotemia refers to elevated BUN, which occurs when the GFR is 20–35% of normal Uremia refers to elevated BUN plus fluid and electrolyte, endocrine, neuromuscular, hematological, or dermatologic, and metabolic abnormalities; it occurs when the patient has overt renal failure and the GFR is <20–25%	
Low level	No symptoms	
After event, time to....		
Initial elevation	Variable, depending on etiology of increase in BUN	
Peak values	Can exceed 100 mg/dL	
Normalization	If prerenal or postrenal etiology of renal failure is corrected, BUN will return to normal range quickly; however, if intrarenal etiology of renal failure results in permanent nephron injury, high levels of BUN may persist; in this case, when uremia develops, the patient may be dialyzed, which will reduce BUN level	
Causes of spurious results	Avoid collecting blood specimens in tubes containing sodium fluoride, which inhibits urease	

BUN = blood urea nitrogen; GFR = glomerular filtration rate; RBCs = red blood cells; SIADH = syndrome of inappropriate antidiuretic hormone.

QUICKVIEW | Creatinine

PARAMETER	DESCRIPTION	COMMENTS
Common reference range		
Adults	0.6–1.2 mg/dL (53–106 µmol/L)	Usually measured with BUN to assess renal function Normal range for serum creatinine varies based on patient age, muscle mass of the patient, and gender; however, usually, the normal range for adults applies to both males and females
Pediatrics	0.2–0.7 mg/dL (18–62 µmol/L)	
Critical value	Variable based on age, race, muscle mass, low-protein diets, and medications	Serum creatinine should not be used alone as an indicator of renal function; an acute decrease in GFR may not be immediately manifested as an increased serum creatinine
Inherent activity	None	
Location		
Production	Creatinine is produced in muscle; it is a waste product of creatine and creatine phosphate	
Storage	Not applicable	
Secretion/excretion	70–80% of creatinine is filtered by the glomerulus, and the rest undergoes tubular secretion via the organic cation pathway	
Causes of abnormal values		
High	Prerenal causes: dehydration, blood loss, shock, congestive heart failure, hypotension, increased intake of meat Intrarenal causes: acute or chronic renal failure due to any cause, glomerulonephritis, acute tubular necrosis, severe hypertension Postrenal causes: obstruction of ureter, bladder neck, or urethra due to stones, enlarged prostate, or stricture, respectively Exposure to nephrotoxins (e.g., aminoglycosides, vancomycin, loop diuretics, penicillinase- resistant penicillins)	
Low	Aging: older patients have less muscle mass; therefore, serum creatinine may be lower than in younger patients Malnourished patients have low muscle mass; therefore, serum creatinine may be decreased	
Signs and symptoms		
High level	If the increased serum creatinine is due to intrinsic renal disease, patients typically will have other laboratory abnormalities; for example, when the GFR is approximately 25 mL/min, increased serum phosphate, uric acid, potassium, and hydrogen ion will result; when the GFR is <10 mL/min, increased sodium and chloride will result	
Low level	Signs and symptoms of the underlying cause of low creatinine levels will be evident (e.g., malnourished patient appears cachectic)	
After event, time to....		
Initial elevation	Variable, depending on the etiology of increase in creatinine; for example, in acute renal failure, serum creatinine often rises within 24–48 hr; after radiocontrast media exposure, serum creatinine rises in 3–5 days; after ischemic renal failure, serum creatinine increases In 7–10 days	
Peak values	Not applicable	
Normalization	If prerenal or postrenal etiology of renal failure is corrected, creatinine will return to normal range quickly; however, if intrarenal etiology of renal failure results in permanent nephron injury, high levels of creatinine may persist; after the cause has been eliminated, the increase in serum creatinine may persist if the patient had irreversible renal dysfunction, or may persist for 7–14 days If the patient had reversible renal dysfunction	

Continued

QUICKVIEW | Creatinine (cont'd)

PARAMETER	DESCRIPTION	COMMENTS
Causes of spurious results	Uric acid, glucose, fructose, acetone, acetoacetate, pyruvic acid, and ascorbic acid, cefoxitin, or flucytosine can cause false elevations in measured creatinine when using the alkaline picrate (Jaffe) assay; this can lead to an underestimation of a patient's creatinine clearance	
	Bilirubin can falsely lower measured creatinine when using the alkaline picrate assay or enzymatic assays; this can lead to an overestimation of a patient's creatinine clearance	
	Cimetidine, trimethoprim, pyrimethamine, dronedarone, or dapsone can compete through the organic cation pathway for tubular secretin of creatinine; these drugs can cause false elevations in serum creatinine	
	Hemolysis of the blood sample can falsely increase creatinine measurement	

BUN = blood urea nitrogen; GFR = glomerular filtration rate.

12

ELECTROLYTES, OTHER MINERALS, AND TRACE ELEMENTS

Lingtak-Neander Chan

OBJECTIVES

After completing this chapter, the reader should be able to

- **Describe the homeostatic mechanisms involved in sodium and water balance, hyponatremia, and hypernatremia**
- **Describe the physiology of intracellular and extracellular potassium regulation as well as the signs and symptoms of hypokalemia and hyperkalemia**
- **List common causes of serum chloride abnormalities**
- **List common conditions resulting in serum magnesium abnormalities and describe signs and symptoms of hypomagnesemia and hypermagnesemia**
- **Describe the metabolic and physiologic relationships among the metabolism of calcium, phosphate, parathyroid hormone, and vitamin D**
- **List common conditions resulting in serum calcium abnormalities and describe signs and symptoms of hypocalcemia and hypercalcemia**
- **List common conditions resulting in altered copper, zinc, manganese, and chromium homeostasis and describe the signs and symptoms associated with their clinical deficiencies**
- **Interpret the results of laboratory tests used to assess sodium, potassium, chloride, calcium, phosphate, magnesium, copper, zinc, manganese, and chromium (in the context of a clinical case description, including history and physical examination)**

Serum or plasma electrolyte concentrations are among the most commonly used laboratory tests by clinicians for assessment of a patient's health status, clinical conditions and disease progression. The purpose of this chapter is to present the physiological basis of the need to assess serum concentrations of common electrolytes and minerals. The interpretation of these laboratory results and the clinical significance of abnormal results are addressed.

Serum sodium, potassium, chloride, and total carbon dioxide content (often referred to *as serum bicarbonate concentration*) are among the most commonly monitored electrolytes in clinical practice. Magnesium, calcium, and phosphate are also monitored as determined by the patient's disease states and clinical indication. The homeostasis of calcium and phosphate is frequently discussed in the context of the endocrine system because of the effects of vitamin D and parathyroid hormone (PTH) on the regulation of these minerals. Serum total carbon dioxide content, often measured in conjunction with electrolytes, is discussed in Chapter 13 because of its significance for the assessment of acid–base status. Listed in **Table 12-1** are the current dietary reference intake for electrolytes, minerals, and trace elements.

TABLE 12-1. Recommended Dietary Reference Intake of Electrolytes and Minerals for Healthy Adults According to the Dietary Guidelines 2015–2020

NUTRIENT	DIETARY REFERENCE INTAKE[a]	
	Male	Female
Sodium	2300 mg (100 mEq)	
Potassium	4700 mg (~120 mEq)	
Chloride	Varies with potassium and sodium intakes	
Magnesium	19–30 yo: 400 mg	19–30 yo: 310 mg
	31+ yo: 420 mg	31+ yo: 320 mg
Calcium	19–70 yo: 1000 mg	19–50 yo: 1000 mg
	71+ yo: 1200 mg	51+ yo: 1200 mg
Phosphorus	700 mg	
Copper	900 mcg	
Zinc	11 mg	8 mg
Manganese	2.3 mg	1.8 mg
Chromium[b]	19–50 yo: 35 mcg	19–50 yo: 25 mcg
	51+ yo: 30 mcg	51+ yo: 20 mcg

yo = years old.

[a] According to the Recommendations from 2015–2020 Dietary Guidelines for Americans. http://health.gov/dietaryguidelines/2015/ (accessed 2016 Feb 8).

[b] Adequate intakes according to Institute of Medicine (U.S.) Food and Nutrition Board. Dietary Reference Intakes. Washington, DC: National Academies Press (U.S.); 2001.

ELECTROLYTES

The traditional units, International System (SI) units, and their conversion factors for electrolytes, minerals, and trace elements discussed in this chapter are listed in **Table 12-2**. Although the normal ranges of serum concentrations for each of the electrolytes are listed below, clinicians should always confirm with the institutional clinical laboratory department for their institutional reference range due to the variance introduced by equipment, analytical technique, and quality assurance data.

Sodium

Normal range: 135–145 mEq/L (135–145 mmol/L)

Sodium is the most abundant cation in the extracellular fluid and is the major regulating factor for bodily fluid and water balance. Extracellular (i.e., intravascular and interstitial) and intracellular sodium contents are closely affected by body fluid status. Thus, an accurate interpretation of serum sodium concentration must include an understanding of body water homeostasis and the interrelationship between the regulation of sodium and water.[1]

Physiology

Sodium is essential for maintaining the optimal transmembrane electric potential for action potential and neuromuscular functioning as well as regulating serum osmolality and water balance. Serum osmolality is an estimate of the water-solute ratio in the vascular fluid. It can be measured in the laboratory or estimated using the following equation:

$$\begin{aligned}&\text{Estimated serum osmolality (mOsm/kg)}\\&= (2 \times \text{serum [Na]}) + [\text{glucose, in mg/dL}]/18\\&\quad + [\text{blood urea nitrogen, in mg/dL}]/2.8\end{aligned}$$

The normal range of serum osmolality is 285–295 mOsm/kg. The measured osmolality should not exceed the estimated value by more than 10 mOsm/kg. A difference of 10 mOsm/kg or more is considered an increased osmolal gap, which may suggest the presence of other unmeasured solutes (e.g., organic solvents, alcohol) and is useful to providing assessments in clinical toxicology. Decreased serum osmolality usually suggests a water excess, whereas increased serum osmolality suggests a water deficit. Although serum osmolality may be helpful in assessing water status, especially the intravascular volume, it should not be the primary and only parameter in assessing fluid status. The results also should be interpreted in the context of the ability of the solute to cross cellular membranes (e.g., uremia causing hyperosmolality without relative intracellular depletion) and the patient's symptoms and signs of disease. **Figure 12-1** summarizes the inter-relationship and regulation between water and sodium.

TABLE 12-2. Conversion Factors to SI Units

NUTRIENT	TRADITIONAL UNITS	CONVERSION FACTORS TO SI UNITS	SI UNITS
Sodium	mEq/L	1	mmol/L
Potassium	mEq/L	1	mmol/L
Chloride	mEq/L	1	mmol/L
Magnesium	mEq/L	0.5	mmol/L
Calcium	mg/dL	0.25	mmol/L
Phosphate	mg/dL	0.3229	mmol/L
Copper	mcg/dL	0.1574	µmol/L
Zinc	mcg/dL	0.153	µmol/L
Manganese	mcg/L	18.2	nmol/L
Chromium	mcg/L	19.2	nmol/L

Changes in body water and plasma volume can directly or indirectly affect the serum sodium concentration. For example, as the result of changes in effective circulating volume, baroreceptors and osmoreceptors will respond accordingly to restore an isovolemic state of the body. Baroreceptors are located in the carotid sinus, aortic arch, cardiac atria, hypothalamus, and the juxtaglomerular apparatus in the kidney. Stimulation of these receptors will promote urinary loss of water and sodium. Osmoreceptors are present primarily in the hypothalamus. The three major effectors in response to the stimulation of the osmoreceptors include vasopressin or antidiuretic hormone (ADH), the renin-angiotensin-aldosterone system, and natriuretic peptides. The resultant renal effects from these three distinct pathways collectively regulate the homeostasis of water and sodium.

The kidneys are the primary organ responsible for the retention and excretion of body sodium and water. The glomeruli receive and filter about 180 L of plasma fluid daily. On average, fewer than 2 L of water and between 0.1–40 g of sodium are excreted in the urine, depending on the fluid status of the individual. Although almost 100% of the plasma sodium is filtered by the glomeruli, <1% is excreted in the urine under normal circumstances. The proximal tubule and the loop of Henle collectively account for up to 90% of sodium reabsorbed from the kidneys.

The homeostatic mechanism for water and sodium also involves the equilibrium among intravascular, interstitial, and intracellular fluids.[3] Net movement of water occurs from areas of low osmolality to areas of high osmolality. This effect can be readily observed in patients with a low serum osmolality due to a deficit of serum sodium or excess of plasma water. In patients with hyponatremia, water moves from the plasma to the higher osmolality in the interstitial space.[3] In the presence of high hydrostatic and oncotic pressure gaps across capillary walls, the net effect is excessive interstitial water accumulation and edema formation.[2,3]

Antidiuretic hormone (vasopressin). ADH, also known as *arginine vasopressin*, is a nine amino acid peptide hormone that regulates renal handling of free water. By altering the amount of water reabsorbed by the kidney, ADH has an indirect but pivotal effect in changing or maintaining serum sodium concentration. ADH is secreted by the magnocellular neurons in the supraoptic and paraventricular nuclei of the hypothalamus, where both osmoreceptors and baroreceptors are present to detect fluid changes in the vasculature.

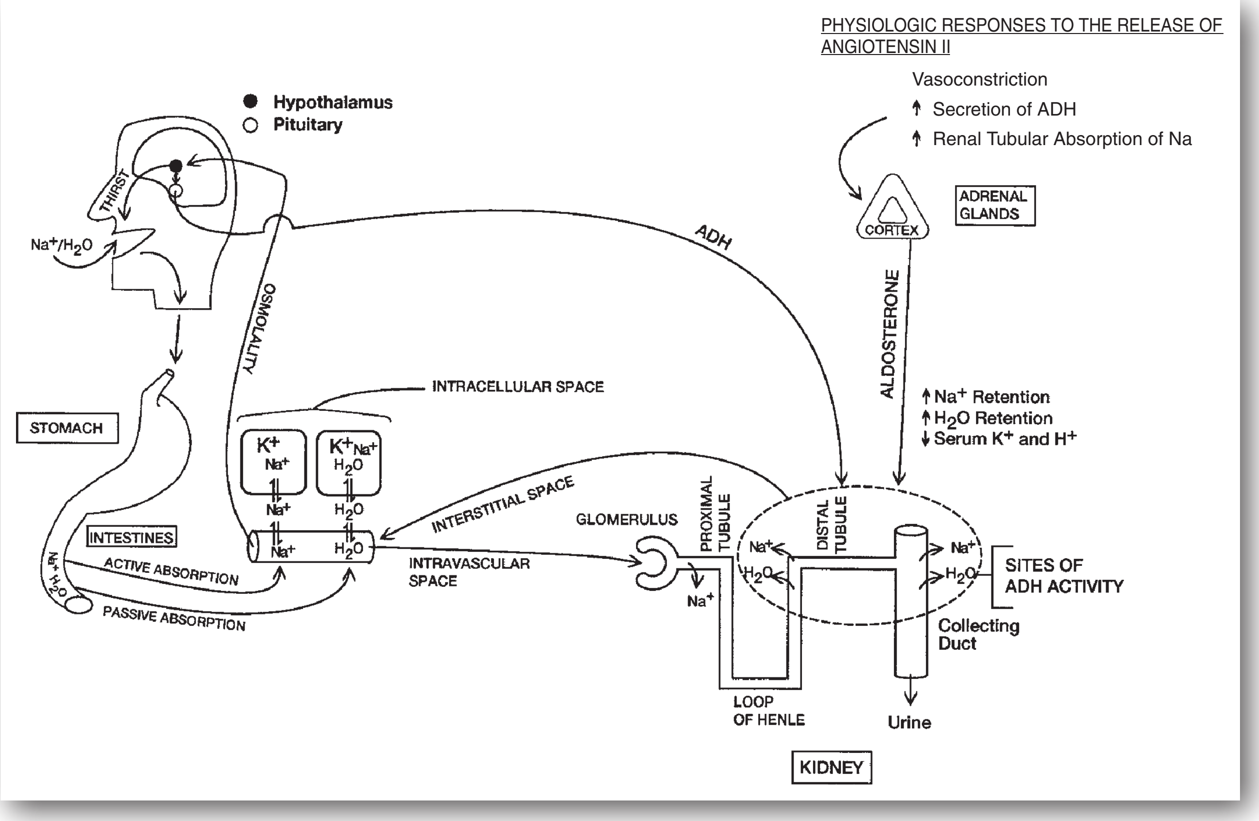
PHYSIOLOGIC RESPONSES TO THE RELEASE OF ANGIOTENSIN II
Vasoconstriction
↑ Secretion of ADH
↑ Renal Tubular Absorption of Na
● Hypothalamus
○ Pituitary
THIRST
Na+/H2O
OSMOLALITY
ADH
ADRENAL GLANDS
CORTEX
ALDOSTERONE
↑ Na+ Retention
↑ H2O Retention
↓ Serum K+ and H+
INTRACELLULAR SPACE
STOMACH
K+ Na+
K+ Na+ H2O
Na+
H2O
INTESTINES
Na+ H2O
ACTIVE ABSORPTION
PASSIVE ABSORPTION
INTERSTITIAL SPACE
INTRAVASCULAR SPACE
GLOMERULUS
PROXIMAL TUBULE
DISTAL TUBULE
Na+
H2O
SITES OF ADH ACTIVITY
Collecting Duct
LOOP OF HENLE
Urine
KIDNEY

FIGURE 12-1. Homeostatic mechanisms involved in sodium, potassium, and water balance. Sodium is the principal cation in blood that contributes to serum osmolality and intravascular volume. When serum osmolality and intravascular volume are increased, baroreceptors in the carotid sinus, aortic arch, cardiac atria, hypothalamus, and juxtaglomerular apparatus in the kidney, promote urinary loss of sodium and water. The synthesis and release of ADH are increased which lead to increased water retention. In addition, elevated serum osmolality with increased serum sodium would suppress aldosterone release, which results in increased renal sodium excretion. In contrast, when serum osmolality is low due to excess intravascular water and decreased sodium, water will passively move from the blood stream to interstitial spaces (resulting in edema) and into cells (in brain cells, this can cause brain swelling). In addition, low serum osmolality detected by hypothalamic osmoreceptors, will suppress release of ADH resulting in decreased water reabsorption in the distal tubule, and stimulate the renin-angiotensin-aldosterone system (which enhances distal tubular reabsorption of sodium and potassium secretion). Serum osmolality also regulates the thirst response. When serum osmolality is high, hypothalamic osmoreceptors stimulate thirst so that the patient increases water intake. Together with increased ADH release the body will increase free water retention and eventually drive serum osmolality back within the normal range.

ADH release is stimulated by (1) hypovolemia (detected by baroreceptors); (2) thirst; (3) increased serum osmolality; and (4) angiotensin II. The plasma half-life of ADH is 10–20 minutes, and it is rapidly deactivated and eliminated by the liver, kidneys, and a plasma enzyme vasopressinase.

ADH regulates urinary water loss by augmenting the permeability of the collecting tubules to increase the net reabsorption of water. Circulating serum ADH binds to type 2 vasopressin (V2) receptors starting at the thick ascending loop, which contributes to the corticomedullary gradient and mechanism of water retention. More importantly, ADH also binds to the V2 receptors in the collecting tubule and promotes the formation of a water channel, known as *aquaporin-2*. Aquaporin-2 facilitates the reabsorption of water from the lumen back into the renal blood supply in the systemic circulation, causing a decrease in diuresis and net retention of water. However, if serum sodium is high but blood volume is normal (e.g., normovolemia with hyperosmolality), the effect from the baroreceptors overrides the further release of ADH, thus preventing volume overload (i.e., hypervolemia).[2]

In patients with the syndrome of inappropriate ADH (SIADH) secretion, an abnormally high quantity of ADH is present in the systemic circulation. This condition results in increased water reabsorption, which could cause a dilutional effect in serum sodium. In conjunction with increased free water intake, a low serum sodium concentration is commonly observed in these patients. Urine osmolality and urine electrolyte concentrations are often increased due to the decreased urinary excretion of free water associated with the increased effect of ADH. Conversely, in patients with central diabetes insipidus (DI), hypothalamic

ADH synthesis or release is decreased. Patients with DI commonly present with hypernatremia due to the increased renal wasting of free water. In some cases, the kidneys fail to respond to the circulating ADH in spite of appropriate synthesis and release of ADH from the hypothalamus. This condition is called *nephrogenic diabetes insipidus*. In either central or nephrogenic DI, patients usually produce a very large quantity of diluted urine, characterized by low specific gravity, low urine osmolality, and low urine sodium.[2] (Chapter 11 offers an in-depth discussion of the effects of other diseases on urine composition.)

Drugs may alter ADH release from the hypothalamus or the biological response to the hormone in the renal epithelial tissues. This may produce an imbalance of water and sodium in the body and exacerbate SIADH or DI.[4,5] SIADH is not uncommon with the chronic use of chlorpropamide, tolbutamide, cyclophosphamide, carbamazepine, oxcarbazepine, some opiate derivatives, oxytocin, vincristine, phenothiazines, some tricyclic antidepressants, and a number of serotonin reuptake inhibitors (**Table 12-3**). Because of their ability to increase renal reabsorption of free water, some of these drugs have an established role in the treatment of chronic hypernatremia or DI. For example, carbamazepine stimulates ADH release and enhances renal cell response to ADH. The dual effects result in a reported incidence of hyponatremia between 5% and 40% associated with its chronic use. But this antidiuretic effect also has established its role as an off-label pharmacotherapeutic option for DI. In contrast, demeclocycline and lithium alter the renal epithelial handling of sodium and water by decreasing the action of ADH, especially on the function and formation of aquaporin. They have been used in the treatment of SIADH. Other drugs that decrease the release and impair the renal response to ADH also may precipitate DI (**Table 12-4**). Based on published data, lithium, foscarnet, and clozapine are the most commonly reported causes of drug-induced DI. In addition, conivaptan, a mixed V1A/V2 antagonist, and tolvaptan, a selective V2 receptor antagonist, both modulate the renal handling of water by reducing renal water absorption and affect sodium homeostasis. These drugs are currently approved for the treatment of euvolemic and hypervolemic hyponatremia.

TABLE 12-3. Medications That Can Cause Hyponatremia Based on Published Data

Drugs That Alter *SODIUM and WATER* Homeostasis:

- Amiloride
- Indapamide
- Loop diuretics
- Thiazide diuretics
- Trimethoprim

Drugs That Alter WATER Homeostasis:

- *Stimulator of central ADH production or release*
  - Antidepressants:
    - Monoamine oxidase inhibitors
    - Selective serotonin reuptake inhibitors
    - Tricyclic antidepressants (more common with amitriptyline, desipramine, protriptyline)
  - Antiepileptic drugs:
    - Carbamazepine
    - Oxcarbazepine
    - Valproic acid
  - Antipsychotic agents:
    - Phenothiazines (e.g., thioridazine, trifluoperazine)
    - Butyrophenones (e.g., haloperidol)
  - Antineoplastic agents:
    - Alkylating agents (cyclophosphamide, ifosphamide, melphalan)
    - Platinum (cisplatin, carboplatin)
    - Vinca alkaloids (more common with vinblastine and vincristine)
    - Others: levamisole, methotrexate
    - Cotrimoxazole (especially at high doses)
    - Opioid analgesics
    - 3,4-methylenedioxymethylamphetamine (MDMA; aka Ecstasy)
- *Enhancers of ADH effect*
  - Antiepileptic drugs (primarily carbamazepine and lamotrigine)
  - Antineoplastic agents (mostly cyclophosphamide)
  - Nonsteroidal anti-inflammatory drugs
  - Oral hypoglycemic agents:
    - Chlorpropamide
    - Tolbutamide
- *Drugs with unclear mechanisms*
  - ACE inhibitors
  - Bromocriptine
  - Oxytocin
  - Venlafaxine

ACE = angiotensin-converting enzyme; ADH = antidiuretic hormone.

Renin-angiotensin-aldosterone system. Renin is a glycoprotein that catalyzes the conversion of angiotensinogen to angiotensin I, which is further converted to angiotensin II primarily in the lungs. However, angiotensin II also can be formed locally in the kidneys. Angiotensin II, a potent vasoconstrictor, is important in maintaining optimal perfusion pressure to end organs especially when plasma volume is decreased. In addition, it induces the release of aldosterone, ADH, and, to a lesser extent, cortisol.

Aldosterone is a hormone with potent mineralocorticoid activity. It affects the distal tubular reabsorption of sodium.[3] This hormone is released from the adrenal cortex. Besides angiotensin II, various dietary and neurohormonal factors including low serum sodium, high serum potassium, and low blood volume also can stimulate its release. Aldosterone acts on renal Na-K-ATPase to increase urinary excretion of potassium from the distal tubules in exchange for sodium reabsorption. Because of its effect on renal Na/K exchange, aldosterone has a profound effect on serum potassium, while its effect on serum sodium is relatively modest. As serum sodium increases, so does water reabsorption, which follows the osmotic gradient.[3] Renal arteriolar blood pressure (BP) then increases, which helps maintain the glomerular filtration rate (GFR). Ultimately,

TABLE 12-4. Drugs That Can Cause Diabetes Insipidus by Decreasing Renal Response to ADH

Precipitant of Nephrogenic DI
Amphotericin B
Cidofovir
Cimetidine
Clozapine
Colchicine
Conivaptan
Cyclophosphamide
Demeclocycline
Epirubicin
Ethanol[a]
Fluvoxamine
Foscarnet
Gentamicin
Lithium
Methicillin[b]
Phenytoin[a] (uncommon at therapeutic doses)
Propoxyphene[b]
Tolvaptan
Verapamil
Vinblastine

ADH = antidiuretic hormone; DI = diabetes insipidus.

[a] Likely also involves central effect by inhibiting ADH release.

[b] Currently no longer available in the United States, although still available in some other countries.

more water and sodium pass through the distal tubules, overriding the initial effect of aldosterone.[2,3]

Natriuretic peptides. Atrial natriuretic factor (ANF), also known as *atrial natriuretic peptide*, is a vasodilatory hormone synthesized and primarily released by the right atrium. It is secreted in response to plasma volume expansion, as a result of increased atrial stretch. ANF inhibits the juxtaglomerular apparatus, zona glomerulosa cells of the adrenal gland, and the hypothalamus-posterior pituitary. As a result, a global down regulation of renin, aldosterone, and ADH, respectively, is achieved. ANF directly induces glomerular hyperfiltration and reduces sodium reabsorption in the collecting tubule. A net increase in sodium excretion is achieved. Therefore, ANF can decrease serum and total body sodium. Brain natriuretic peptide (BNP) is produced and secreted primarily by the ventricles in the brain, and to a much smaller extent, the atrium. Similar to ANP, BNP also regulates natriuretic, endocrine, and hemodynamic responses and may affect sodium homeostasis. An increase in blood volume or pressure, such as chronic heart failure and hypertension, enhances BNP secretion, which induces a significant increase in natriuresis and to a lesser extent, urinary flow (i.e., diuresis). Plasma BNP concentrations correlate with the magnitude of left ventricular heart failure and the clinical prognosis of patients with heart failure.

Hyponatremia

Hyponatremia is defined as a serum sodium concentration <135 mEq/L (<135 mmol/L). Although it can be the direct result of sodium deficit, hyponatremia may also occur when total body water content is low (i.e., dehydration), normal, or high (i.e., fluid overload). Therefore, natremic status must be evaluated in concert with volume status to determine the nature of an underlying disorder. Fluid status should be evaluated based on history of oral intake, vital signs; other supportive laboratory findings if available (e.g., serum blood urea nitrogen (BUN)–serum creatinine (SCr) ratio, hematocrit to hemoglobin (Hgb) concentration ratio, or urine electrolyte assessment); recent changes in body weight; recent medical, surgical, and nutrition history; and findings from the physical examination. More importantly, the patient's renal function, hydration status, and fluid intake and output must be thoroughly evaluated and closely monitored. The most common causes of hyponatremia can be broken down into two types: (1) sodium depletion in excess of total body water loss (e.g., severe dehydration with true depletion of total body sodium); or (2) dilutional hyponatremia (i.e., free water intake greater than water output with no change in sodium loss). Dilutional hyponatremia can be further categorized into five subtypes: (1) primary dilutional hyponatremia (e.g., SIADH and renal failure); (2) neuroendocrine (e.g., adrenal insufficiency and myxedema); (3) psychiatric disorder (e.g., psychogenic polydipsia); (4) osmotic hyponatremia (e.g., severe hyperglycemia); and (5) thiazide diuretic-induced.

Most patients with hyponatremia remain asymptomatic until serum sodium approaches 120 mEq/L. Infusion of hypertonic saline (e.g., 3% NaCl solution) is usually not necessary unless serum sodium concentration is <120 mEq/L, altered mental status is present, or if the patient is fluid restricted (e.g., heart failure, chronic renal failure). As with most electrolyte disorders, the chronicity of the imbalance is a major determinant of the severity of signs and symptoms. For example, hyponatremia in patients with congestive heart failure (CHF) secondary to chronic, progressive volume overload and decreased renal perfusion is less likely to be symptomatic than a patient who is hyponatremic due to rapid infusion of a hypotonic solution. The most commonly reported symptom associated with hyponatremia is altered mental status (**Table 12-5**). If serum sodium continues to fall, cerebral edema can worsen and intracranial pressure will continue to rise. More severe symptoms such as seizure, coma, and, subsequently, death may result.[2-6]

Hyponatremia associated with total body sodium depletion. Hyponatremia associated with low total body sodium reflects a reduction in total body water, with an even larger reduction in total body sodium. This condition is primarily caused by depletion of extracellular fluid, which stimulates ADH release to increase renal water reabsorption even at the expense of causing a transient hypo-osmotic state. Some common causes include vomiting; diarrhea; intravascular fluid losses due to burn injury and pancreatitis; Addison disease; and certain forms of renal failure (e.g., salt-wasting nephropathy).[2] This type of hyponatremia may also occur in patients treated too

TABLE 12-5. Signs and Symptoms of Hyponatremia

Agitation
Anorexia
Apathy
Depressed deep-tendon reflexes
Disorientation
Headache
Hypothermia
Lethargy
Muscle cramps
Nausea
Seizures
Vomiting

aggressively with diuretics who receive sodium-free solutions as replacement fluid.

Hyponatremia associated with normal total body sodium. Also called *euvolemic* or *dilutional hyponatremia*, this condition refers to impaired free water excretion without alteration in sodium excretion. Etiologies include any mechanism that enhances ADH secretion or potentiates its action at the renal collecting tubules. This condition can occur as a result of glucocorticoid deficiency, severe hypothyroidism, and administration of water to a patient with impaired water excretion capacity.[2,5] SIADH is associated with an excessive renal reabsorption of free water in the body due to continued ADH secretion despite low serum osmolality. This results in hyponatremia and increased urinary sodium loss. Patients with SIADH produce concentrated urine with high urine osmolality (usually >200 mOsm/kg H_2O) and urine sodium excretion (as reflected in a urine sodium concentration that is usually >20 mEq/L). They have normal renal, adrenal, and thyroid function and have no evidence of volume abnormalities.[25]

Impaired ADH response can be precipitated by many factors, including medications. SIADH has been reported in patients with certain tumors, such as lung cancer, pancreatic carcinoma, thymoma, and lymphoma. ADH release from the parvicellular and magnocellular neurons may be stimulated by cytokines such as interleukin (IL-2, IL-6, IL-1 β), and tumor necrosis factor (TNF-α). Likewise, head trauma, subarachnoid hemorrhage, hydrocephalus, Guillain-Barré syndrome, pulmonary aspergillosis, and occasionally tuberculosis may increase hypothalamic ADH production and release leading to SIADH (**Figure 12-2**).

In some cases, hyponatremia may not be associated with a sodium deficit. This scenario is associated with normal or even slightly elevated total body sodium, which is distributed in a much larger volume of total body water. It is frequently observed in hypervolemic states with compromised renal function such as CHF, cirrhosis, nephrotic syndrome, and chronic kidney disease (CKD). In these patients, renal handling of water and sodium is often impaired.[2,5]

The initial goal of therapy for most patients with hyponatremia is to raise the serum sodium concentration to 130 mEq/L over a few days. Mild, asymptomatic hyponatremia (>125 mEq/L) can usually be safely managed with a sodium-containing oral rehydration solution or an increase in oral sodium intake, provided that the oral route is viable (i.e., vomiting and diarrhea are controlled, evidence of functional intestine). Intravenous (IV) sodium therapy is preferred in more severe cases of hyponatremia. In most cases, sodium chloride 0.9% is used. If a hypertonic saline solution (e.g., NaCl 3% or higher) is used, it must be infused via a central venous catheter due to its high osmolarity.

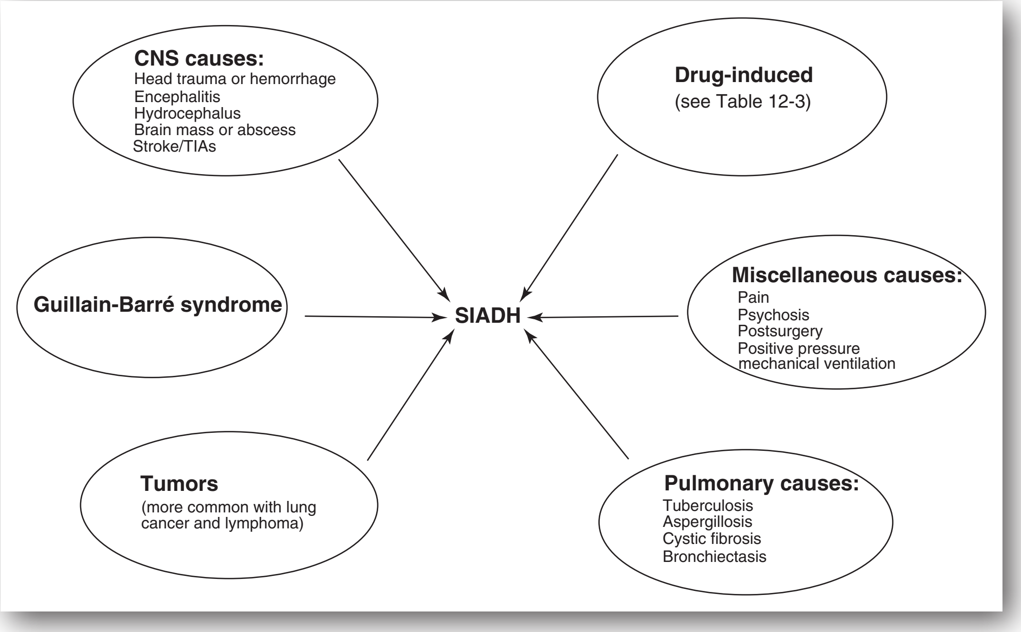
CNS causes:
Head trauma or hemorrhage
Encephalitis
Hydrocephalus
Brain mass or abscess
Stroke/TIAs
Drug-induced
(see Table 12-3)
Guillain-Barré syndrome
SIADH
Miscellaneous causes:
Pain
Psychosis
Postsurgery
Positive pressure mechanical ventilation
Tumors
(more common with lung cancer and lymphoma)
Pulmonary causes:
Tuberculosis
Aspergillosis
Cystic fibrosis
Bronchiectasis

FIGURE 12-2. Etiologies of SIADH. TIAs = transient ischemic attacks.

The initial goal for treating hyponatremia is to increase serum sodium by 4–6 mEq/L within 24 hours of baseline in most cases. Neurological deficits would improve with this target rate of change in serum sodium concentration. The average rate of increase in serum sodium should not exceed 1–2 mEq/L/hr and 9 mEq/L in any given 24-hour period. There is no evidence that correction of serum sodium by >10 mEq/L in 24 hours or 18 mEq/L in 48 hours improves the outcomes in patients with acute or chronic hyponatremia. (**Minicase 1**.)

Tests for Assessing Fluid Status

Fractional Excretion of Sodium

Normal range: 1–2%

In most cases, natremic disorders cannot be effectively managed without first optimizing the overall fluid status of the patient. Therefore, when a serum sodium value is abnormal, the clinician should first evaluate whether vascular volume is optimal. In addition to physical examinations and history, the *fractional excretion of sodium* (FE_{Na}) may help validate these findings, especially in patients whose physical examination results may be limited by other confounders (e.g., the use of antihypertensive drugs, heart failure, or with acute renal failure). The FE_{Na} may be determined by the use of a random urine sample to determine renal handling of sodium. FE_{Na}, the measure of the percentage of filtered sodium excreted in the urine, can be calculated using the following equation:

$$FE_{Na} = 100 \times \frac{\text{sodium}_{\text{urinary}} \times \text{creatinine}_{\text{plasma}}}{\text{sodium}_{\text{plasma}} \times \text{creatinine}_{\text{urinary}}}$$

Values >2% usually suggest that the kidneys are excreting a higher than normal fraction of the filtered sodium, implying likely renal tubular damage. Conversely, FE_{Na} values <1% generally imply preservation of intravascular fluid through renal sodium retention, suggesting prerenal causes of renal dysfunction (e.g., hypovolemia and cardiac failure). Because acute diuretic therapy can increase the FE_{Na} to 20% or more, urine samples should be obtained at least 24 hours after diuretics have been discontinued.[2]

Blood Urea Nitrogen: Serum Creatinine Ratio

Normal range: <20:1

The *BUN: SCr ratio* can provide useful information to assess fluid status. When this ratio is higher than 20:1, dehydration is usually present. As extracellular fluid volume is diminished, the rate of increase in serum urea is much faster than that with SCr. Therefore, BUN increases by a larger magnitude

MINICASE 1

A Case of Hyponatremia

Jessica F., a 24-year-old woman, presents to the emergency department with lower abdominal pain, fatigue, headache, and dizziness. She had four episodes of vomiting and six episodes of diarrhea in the last 24 hours. She had salad at a salad bar for lunch the day before. About two hours after her lunch, she started to feel nauseated. The abdominal pain and vomiting started shortly after and the diarrhea started in the evening. She vomited her lunch, and her diarrhea was mostly watery without blood. She also experienced headache this morning. She has not been eating for the last 24 hours and can tolerate only small sips of water.

Upon presentation, she looks pale with sunken eyes. She is alert and oriented to time, person, and place. Neurological examination reviews no deficits. Her vital signs include BP 105/70 mm Hg at supine position (standing BP 90/65 mm Hg), HR 92 beats/min (standing 108 beats/min), and RR 20 breaths/min. She also has a fever at 100.6 °F. Blood work for serum electrolyte and complete blood count are ordered. Her electrolyte panel shows the following results: sodium 128 mEq/L, potassium 3.3 mEq/L, chloride 90 mEq/L, CO_2 content 21 mEq/L, BUN 28 mg/dL, creatinine 1 mg/dL, and glucose 77 mg/dL. She does not take any medication prior to this admission.

QUESTION: How would you interpret this patient's serum sodium concentration?

DISCUSSION: The patient's serum sodium concentration is lower than the normal range, suggesting hyponatremia. However, as mentioned above, sodium disorder cannot be fully assessed without evaluating a person's fluid status. Based on the history, she had excessive fluid loss due to repeated episodes of vomiting and diarrhea. Fever also will increase insensible fluid loss. Therefore, she is likely dehydrated (hypovolemic). Her vital signs (orthostatic hypotension with reflex tachycardia), and the findings from physical exam support dehydration. The laboratory results show an elevated BUN:SCr ratio of 28:1, which also is consistent with volume depletion. Increased loss of body fluids, especially from the GI tract, will lead to increased sodium loss. Her fluid intake has been very limited and likely inadequate to replenish the continued sodium loss, which results in hyponatremia. She is likely experiencing hyponatremia associated with total sodium deficiency due to uncontrolled vomiting, diarrhea, and insufficient oral intake.

The onset of the patient's hyponatremia is likely acute because there are no other established factors that would lead to chronic hyponatremia (e.g., use of diuretic drugs, selective serotonin reuptake inhibitors, etc.). Her symptoms of hyponatremia are mild as she shows no neurological deficit. Her headache is likely associated with her dehydration, mild hyponatremia, and possibly acid–base changes.

In summary, this patient has mild hyponatremia with hypovolemia. The cause seems to be from her acute illness—uncontrolled vomiting and diarrhea lead to increase sodium loss with insufficient sodium intake. She does not seem to experience major acute symptoms associated with hyponatremia at this point. The logical treatment approach for her will involve controlling her nausea, diarrhea, and vomiting, as well as treating hypovolemia with a sodium containing fluid (e.g., NaCl 0.9%) and managing other electrolyte disturbances.

than the SCr concentration in dehydrated individuals, leading to a rise in the BUN: SCr ratio. However, it should be noted that BUN will increase in the face of internal bleeding, CHF, renal failure, or significantly increased protein intake. If any of these conditions are present, additional signs and symptoms of dehydration should be assessed along with the increased BUN:SCr ratio.

Hypernatremia

Hypernatremia is defined as a serum sodium concentration >145 mEq/L (>145 mmol/L). High serum sodium concentrations are common in patients with either an impaired thirst expression (e.g., neurohypophyseal lesion, especially after suffering from a stroke) or an inability to replete water deficit through normal insensible losses (i.e., uncontrollable water loss through respiration or skin) or from renal or GI losses. All hypernatremic states increase serum osmolality. Similar to hyponatremia, hypernatremia may occur in the presence of high, normal, or low total body water content.[2,3,6]

The clinical manifestations of hypernatremia primarily involve the neurological system. These manifestations are the consequence of dehydration, particularly in the brain. In adults acute elevation in serum sodium above 160 mEq/L (>160 mmol/L) is associated with a 75% mortality rate. Unfortunately, neurological sequelae are common even in survivors. To assess the etiology of hypernatremia, it is important to determine (1) urine production; (2) sodium intake; and (3) renal solute concentrating ability, which reflects ADH activity.

Hypernatremia associated with low total body water occurs when the loss of water exceeds the loss of sodium.[3] The thirst mechanism generally increases water intake, but this adjustment is not always possible (e.g., institutionalized elderly patients). This condition also may be iatrogenic when hypotonic fluid losses (e.g., profuse sweating and diarrhea) are replaced with an excessive amount of salt-containing fluids. In these circumstances, fluid loss should be replaced with IV dextrose solutions or hypotonic saline solutions, which serve as a source of free water.[3,5] In hypernatremic patients presenting with high urine osmolality (>800 mOsm/L, roughly equivalent to a specific gravity of 1.023) and low urine sodium concentrations (<10 mEq/L), these laboratory results reflect an intact renal concentrating mechanism. Signs and symptoms of dehydration should be carefully examined. These include orthostatic hypotension, flat neck veins, tachycardia, poor skin turgor, and dry mucous membranes. In addition, the BUN: SCr ratio may be >20 secondary to dehydration.[2,5]

Hypernatremia may be associated with normal total body water, also known as *euvolemic hypernatremia*. This condition refers to an increased loss of free water without concurrent sodium loss.[2] Because of water redistribution between the intracellular and extracellular fluid, no plasma volume contraction is usually evident unless water loss is substantial. Etiologies include increased insensible water loss (e.g., fever, extensive burns) and central and nephrogenic DI. The clinician should be aware of drugs that may cause nephrogenic DI (Table 12-5).[2,5]

Free water supplementation by mouth or IV fluid administration with dextrose 5% is necessary for correcting hypernatremia and preventing hypovolemia. If the diagnosis of DI is subsequently established, vasopressin or desmopressin, a synthetic analog of vasopressin, will be a reasonable option for long-term maintenance therapy.

Hypernatremia also may be associated with high total body water. This form of hypernatremia is the least common because sodium homeostasis is maintained indirectly through the control of water, and defects in the system usually affect total body water more than total body sodium.[3] This form of hypernatremia usually results from exogenous administration of solutions containing large amounts of sodium:

- Resuscitative efforts using hypertonic sodium bicarbonate
- Inadvertent IV infusion of hypertonic saline solutions (i.e., solutions >0.9% sodium chloride)
- Inadvertent dialysis against high sodium-containing solutions
- Sea water, near drowning

Primary hyperaldosteronism and Cushing syndrome may also cause this form of hypernatremia. Large quantities of sodium can be found in the urine of these patients. Signs and symptoms include diminished skin turgor and elevated plasma proteins.[3,5] (**Minicase 2**.)

Potassium

Normal range: 3.8–5.0 mEq/L (3.8–5.0 mmol/L)

Potassium is the primary cation in the intracellular space, with an average intracellular fluid concentration of about 140 mEq/L (140 mmol/L). The major physiological role of potassium is in the regulation of muscle and nerve excitability. It may also play important roles in the control of intracellular volume (similar to the ability of sodium in controlling extracellular volume), protein synthesis, enzymatic reactions, and carbohydrate metabolism.[7,8]

Physiology

The most important aspect of potassium physiology is its effect on action potential, especially on muscle and nervous tissue excitability.[2] During periods of potassium imbalance, the cardiovascular system is of principal concern. Cardiac muscle cells depend on their ability to change their electrical potentials, with accompanying potassium flux when exposed to the proper stimulus, to result in muscle contraction and nerve conduction.[7,8] One important aspect of potassium homeostasis is its distribution equilibrium. In a 70-kg man, the total body potassium content is about 4000 mEq. Of that amount, only a small fraction (about 60 mEq) is distributed in the extracellular fluid; the remainder resides within cells. The average daily Western diet contains 50–100 mEq of potassium, which is completely and passively absorbed in the upper gastrointestinal (GI) tract. To enter cells, potassium must first pass through the extracellular compartment.

If the serum potassium concentration rises above 6 mEq/L (>6 mmol/L), symptomatic hyperkalemia is expected. Potassium homeostasis is altered by insulin, aldosterone, changes in acid–base balance, renal function, or GI and skin losses.

MINICASE 2

A Case of Hypernatremia After Resection of Pituitary Tumor

Theresa L., a 49-year-old woman with a recently diagnosed pituitary tumor, has been admitted to the hospital two days ago for tumor resection. On postoperative day 2, she complains that she feels thirsty and a little dizzy. The nurse reports that she has been asking for water throughout the morning. She also has used the bathroom four times this morning.

Current vital signs: SBP/DBP 108/80 mm Hg supine; 105/80 mm Hg lying down; HR 84–90 beats/min; RR 10–14 breaths/min; SpO_2 (saturation of peripheral oxygen via pulse oximetry) 99% on room air; breathing comfortably. Intake (last 24 hours): five 8-oz glasses of water, 200 mL juice from breakfast, and 2 cups of hot tea.

Output: 3130 mL of urine in the last 16 hours; weight: 63.7 kg today (64.2 kg yesterday; 64.7 kg preoperative).

Laboratory results: sodium 155 mEq/L, potassium 3.2 mEq/L, chloride 101 mEq/L, BUN 18 mg/dL, CO_2 content 24 mEq/L, creatinine 1 mg/dL, glucose 72 mg/dL. Urine osmolality 105 mOsm/kg H_2O; urine specific gravity 1.001.

QUESTION: How would you interpret this patient's serum sodium concentration?

DISCUSSION: The patient's serum sodium concentration is elevated, suggesting hypernatremia. The next step is to assess her fluid status and determine the cause(s) of the disorder(s). Based on the history, she has an unusually high urine output (over 3 L in 16 hours and frequent urination). Her urine osmolality and specific gravity show that she has diluted urine. This suggests that an excessive loss of free water likely has contributed to her hypernatremia. She is currently not dehydrated (based on her vital signs, BUN–SCr ratio) because she has been able to catch up with her urinary fluid loss with oral fluid Intake due to thirst. Her weight change suggests that she is trending toward a mild fluid deficit. Thus, she can be described as having normovolemic hypernatremia.

The onset of her hypernatremia is likely acute because it occurred within two days after her surgery. Her symptom of hypernatremia is limited to dizziness. Surgical procedures that could potentially affect pituitary gland functions are a major risk factor for sodium disorders because the release and regulation of ADH may be affected. In this patient's case, the supraopticohypophyseal tract was likely affected during removal of the tumor and this precipitated the symptoms and signs that are currently observed. The elevated urine output with persistent thirst suggests that an ADH-related disorder, DI, is likely present with a serious risk of altered sodium homeostasis. Her relatively normal vital signs were maintained by her ability to temporarily increase oral fluid intake. But if the DI defect is not corrected, she will develop hypovolemia very quickly. This is an acute medical problem, and the diagnosis should be established quickly with the help of several laboratory tests such as urine sodium, serum sodium, and urine osmolality.

In summary, this patient has hypernatremia, which appears to be manifested by altered renal water/salt regulation based on the urine electrolyte and osmolality. Although her volume status appears normal at this point, she can quickly develop hypovolemia if she is unable to keep up with the oral fluid intake. The cause of hypernatremia is likely related to the pituitary gland resection that caused DI. The logical treatment approach for her will involve free water provision to prevent free water deficit, as well as treating DI. If her oral water intake is unable to match the urinary water loss, she will require concurrent IV fluid therapy to prevent severe dehydration.

These conditions can be modulated by various pathological states as well as pharmacotherapy. Although potassium may affect different bodily functions, its effect on cardiac muscle is by far the most important clinical monitoring parameter. Life-threatening arrhythmias may result from either high or low serum potassium concentrations.[3,6-10]

Renal Homeostasis

When the serum potassium concentration is high, the body has two different mechanisms to restore potassium balance. One quick way is to shift the plasma potassium into cells, while the other slower mechanism is renal elimination.[10] The kidneys are the primary organs involved in the control and elimination of potassium. Potassium is freely filtered at the glomeruli and almost completely reabsorbed before the filtrate reaches the collecting tubules. However, an amount equal to about 10% of the filtered potassium is secreted into the urine at the distal and collecting tubules. Virtually all the potassium recovered in urine is, therefore, delivered via tubular secretion rather than glomerular filtration.[7]

In the distal tubule, potassium is secreted into the tubule, while sodium is reabsorbed. There are several mechanisms that can modulate this sodium–potassium exchange. Aldosterone plays an important role because it increases potassium secretion into the urine (**Figures 12-1** and **12-3**).[10] The hormone is secreted by the adrenal glands in response to high serum potassium concentrations. The delivery of large quantities of sodium and fluid to the distal tubules may also cause potassium secretion and its subsequent elimination, as seen in diuretic-induced hypokalemia.[11] As the delivery of sodium and fluid is decreased, potassium secretion declines.

The presence of other anions in the distal tubules can increase renal potassium loss because the negatively charged anions attract positively charged potassium ions. This mechanism is responsible for hypokalemia caused by renal tubular acidosis and the administration of high doses of drugs as sodium salts (e.g., sodium penicillin, disodium ticarcilin.[10] Potassium secretion also is influenced by the potassium concentration in distal tubular cells. When the intracellular potassium concentration is high, such as during dehydration,

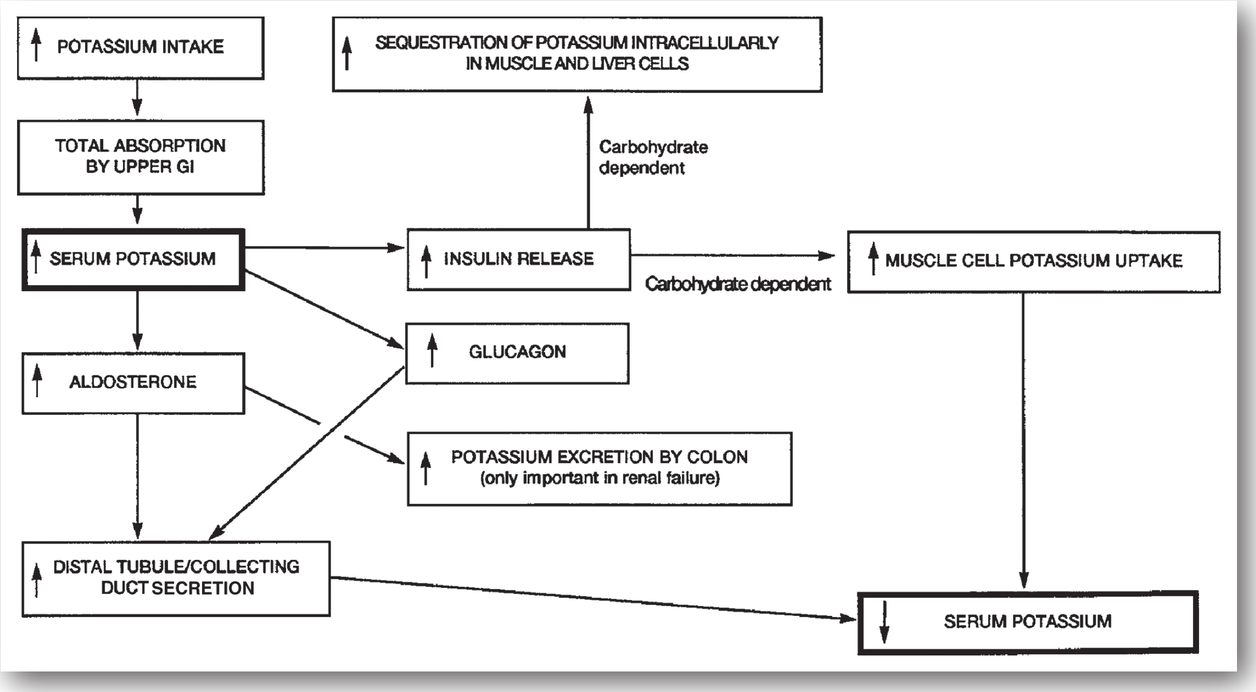
↑ POTASSIUM INTAKE
TOTAL ABSORPTION BY UPPER GI
↑ SERUM POTASSIUM
↑ ALDOSTERONE
↑ DISTAL TUBULE/COLLECTING DUCT SECRETION
↑ SEQUESTRATION OF POTASSIUM INTRACELLULARLY IN MUSCLE AND LIVER CELLS
Carbohydrate dependent
↑ INSULIN RELEASE
Carbohydrate dependent
↑ MUSCLE CELL POTASSIUM UPTAKE
↑ GLUCAGON
↑ POTASSIUM EXCRETION BY COLON (only important in renal failure)
↓ SERUM POTASSIUM

FIGURE 12-3. **The acute homeostatic sequence of events in the body to maintain serum potassium within a narrow concentration range. An increased serum potassium will trigger an increase in aldosterone secretion, which increases distal tubular potassium secretion. In patients with hyperkalemia and renal failure, aldosterone will increase colonic potassium excretion. In patients with hyperkalemia and hyperglycemia, administration of insulin can shift potassium from the intravascular space into cells.**

potassium secretion into the urine is increased. The modulation of renal potassium excretion by these mechanisms may take hours to correct a serum potassium concentration, even during drastic, acute changes. Extrarenal mechanisms, therefore, often play important roles in keeping the serum potassium concentration within the narrow acceptable range. Although the kidneys are the primary route of elimination, potassium secretion into the colon becomes important in patients with advanced renal failure.[10]

Acid–Base Homeostasis

Another potentially relevant factor influencing renal potassium secretion is serum pH. When arterial pH increases due to metabolic alkalosis, a compensatory efflux of hydrogen ions from the cells into the extracellular fluid (bloodstream) takes place with a concurrent influx of potassium ions into the cells to maintain an electropotential gradient.[7] During the early phase of metabolic alkalosis, the serum potassium concentration is transiently reduced due to a pH-dependent intracellular influx of serum potassium from the serum without altering the total body amount. Thus, although there is no immediate change in the amount of total body potassium, this movement of ions increases the cellular potassium content and results in hypokalemia. However, a shift in potassium and hydrogen ions also takes place in the renal distal tubular cells. In the presence of persistent alkalemia, renal potassium secretion into the urine is increased. Over time, the serum potassium concentration declines through increased renal loss, resulting in a reduced body store.

Metabolic acidosis has the opposite effect. Decreased pH results in an extracellular shift of potassium as a result of an intracellular shift of hydrogen ions, causing an elevated serum potassium concentration.[7] Because the intracellular potassium content of the distal tubular cell is decreased, secretion of potassium in the urine is diminished. Chronically, however, renal potassium loss gradually increases due to unknown mechanisms.

When a severe metabolic acid–base abnormality exists, adjustment of the measured serum potassium concentration may be necessary to more accurately assess body potassium status. For every 0.1 unit reduction in arterial pH from 7.4, roughly 0.6 mEq/L (range: 0.2–1.7 mEq/L) could be added to the serum potassium value:

$$K_{corr} = ([7.4 - pH]/0.1 \times 0.6 \text{ mEq/L}) + K_{uncorr}$$

where K_{corr} is the corrected serum potassium concentration and K_{uncorr} is the uncorrected or measured serum potassium concentration.[7] It is important to note that K_{corr} is a hypothetical value and only reflects what the serum potassium concentration would be if the serum pH is normalized and in the absence of other factors affecting potassium homeostasis. As long as the serum pH remains abnormal, the measured serum potassium concentration (K_{uncorr}) is the true reflection of actual serum potassium concentration. The K_{corr} value should always be assessed together with the actual serum potassium concentration and the patient's clinical presentation. The clinical value of calculating K_{corr} is mostly to avoid overcorrection of potassium based solely on K_{uncorr}, as well as to provide a more complete picture that reflects total potassium stores in the body. Clinicians should remember that regardless of the value of K_{corr}, a patient with a significantly abnormal measured (uncorrected) serum potassium concentration is still at risk for developing cardiac arrhythmias.

Acute Homeostasis

Figure 12-3 summarizes the acute homeostatic mechanism involved in potassium distribution. During hyperkalemia, along with the release of aldosterone, increased glucagon and insulin release also contribute to reducing the serum potassium concentration. Glucagon stimulates potassium secretion into the distal tubules and collecting ducts, while insulin promotes intracellular potassium uptake. Although insulin is not a major controlling factor in potassium homeostasis, it is useful for the emergency treatment of hyperkalemia.[7,12]

Pharmacological stimulation of β-2 adrenergic receptors may also affect the transcellular equilibrium of potassium. It leads to the movement of potassium from extracellular fluid to the intracellular fluid compartment. Therefore, β-2 adrenergic agonists (e.g., albuterol) can be used short term to treat certain hyperkalemic patients.[7-9]

Hypokalemia

Hypokalemia is defined as a serum potassium concentration <3.8 mEq/L (<3.8 mmol/L).[10] To interpret the significance of low potassium values, clinicians should determine whether hypokalemia is due to intracellular shifting of potassium (apparent deficit) or increased loss from the body (true deficit) (**Table 12-6**). Intracellular shifting occurs as a result of metabolic alkalosis, after administration of insulin, or giving large doses of β-2 adrenergic agonists (e.g., continuous or hourly use of albuterol in ICU patients receiving mechanical ventilation).[9,13] Increased elimination of potassium can occur in the kidneys or GI tract. There may be decreased potassium reabsorption in the proximal tubules or increased secretion in the distal tubules and collecting ducts.[10]

Amphotericin B. Proximal tubular damage can occur with amphotericin B therapy, resulting in renal tubular acidosis. Amphotericin B directly impairs the reabsorption of potassium, magnesium, and bicarbonate and leads to hypokalemia, hypomagnesemia, and metabolic acidosis.[7,13] A concurrent deficiency in magnesium may affect the ability to restore potassium balance. Magnesium functions as a cofactor to maintain the sodium–potassium adenosine triphosphate (ATP) pump activity and facilitates renal preservation of potassium. A patient with concurrent hypokalemia and hypomagnesemia will not respond to potassium replacement therapy effectively unless magnesium balance is restored.[7,8,12] Lipid formulations of amphotericin B may still affect potassium homeostasis, although the magnitude may be less severe and the presentation is less acute.

TABLE 12-6. Etiologies of Hypokalemia

- Apparent deficit—intracellular shifting of potassium
  - Alkalemia
  - β-2 adrenergic stimulation
  - Insulin (more common with IV bolus or infusion)
- True deficit
  - Decreased intake
    - Alcoholism
    - Potassium-free IV fluids
    - Anorexia nervosa
    - Bulimia
  - Increased output (extrarenal)
    - Vomiting
    - Diarrhea
    - Laxative abuse
    - Intestinal fistulas
    - Renal loss
    - Corticosteroids- especially fludrocortisone and hydrocortisone
    - Amphotericin B
    - Loop and thiazide diuretics
    - Hyperaldosteronism
    - Cushing syndrome
    - Licorice ingestion
    - Patiromer

IV = intravenous.

Diuretics. Nonpotassium-sparing diuretic agents are drugs most commonly associated with renal potassium wasting. Although their mechanisms of natriuretic action differ, diuretic-induced hypokalemia is primarily caused by increased secretion of potassium at the distal sites in the nephron in response to an increased load of exchangeable sodium. Diuretics increase the distal urinary flow by inhibiting sodium reabsorption. This increased delivery of fluid and sodium in the distal segment of the nephron results in an increase in sodium reabsorption at that site. To maintain a neutral electropotential gradient in the lumen, potassium is excreted as sodium is reabsorbed. Therefore, any inhibition of sodium absorption by diuretics proximal to or at the distal tubules can increase potassium loss. Renal potassium excretion is further enhanced when nonabsorbable anions are present in the urine.

Loop diuretics (e.g., furosemide) or thiazides (e.g., hydrochlorothiazide) are associated with hypokalemia and the effect is dose-dependent. Serum potassium concentrations should be monitored regularly, especially in patients receiving high doses of loop diuretics, to avoid the increased risk of cardiovascular events secondary to hypokalemia and other electrolyte imbalances. In addition, elderly patients with ischemic heart disease and patients receiving digoxin are more susceptible to the adverse consequences of hypokalemia.[15-17] Other drugs commonly used in managing hypertension and other cardiac diseases such as spironolactone, triamterene, amiloride, eplerenone, angiotensin-converting enzyme (ACE) inhibitors, and angiotensin receptor antagonists are not expected to cause potassium loss due to their mode of action. On the contrary, they cause retention of potassium due to their effects related to aldosterone-dependent exchange sites in the collecting tubules.[11,18]

Other causes. Conditions that cause hyperaldosteronism, either primary (e.g., adrenal tumor) or secondary (e.g., renovascular hypertension), can produce hypokalemia.[13] Cushing syndrome leads to increased circulation of mineralocorticoids such as aldosterone. Corticosteroids with strong mineralocorticoid activity (e.g., fludrocortisone and hydrocortisone) also can cause hypokalemia.[10]

GI loss of potassium can be important. Aldosterone influences both renal and intestinal potassium handling.[10] A decrease in extracellular volume increases aldosterone secretion, which promotes renal and colonic potassium wasting. The potassium concentration in the GI fluid varies depending on the location of the GI tract ranging from 5 mEq/L (bile, duodenum) to 30 mEq/L (colon). Therefore, profuse and uncontrolled diarrhea can result in potassium depletion. In contrast, upper GI secretion contains a much lower amount of potassium, and loss secondary to vomiting is unlikely to be significant. However, with severe vomiting, the resultant metabolic alkalosis may lead to hypokalemia due to intracellular shifting of potassium and enhanced urinary elimination. Finally, patients receiving potassium-free parenteral fluids can develop hypokalemia if not monitored properly.[7,10]

Clinical diagnosis. Signs and symptoms of hypokalemia involve many physiological systems. Abnormalities in the cardiovascular system may result in serious consequences (i.e., disturbances in cardiac rhythm). Hypokalemia-induced arrhythmias are of particular concern in patients receiving digoxin. Both digitalis glycosides and hypokalemia inhibit the sodium–potassium ATP pump in the cardiac cells. Together, they can deplete intracellular potassium, which may result in fatal arrhythmias. The signs and symptoms of hypokalemia are listed in **Table 12-7**. Skeletal muscle weakness is often seen; severe depletion may lead to decreased reflexes and paralysis. Death can occur from respiratory muscle paralysis.[7,9,19]

Hyperkalemia

Hyperkalemia is defined as a serum potassium concentration >5 mEq/L (>5 mmol/L). As with hypokalemia, hyperkalemia may indicate a true or apparent potassium imbalance, although the signs and symptoms are indistinguishable.[10] To interpret a high serum potassium value, the clinician should determine whether hyperkalemia is due to apparent excess caused by extracellular shifting of potassium or true potassium excess in the body caused by increased intake with diminished excretion (**Table 12-8**).[4,6,9,11]

Causes. Because renal excretion is the major route of potassium elimination, renal failure is the most common cause of hyperkalemia. However, potassium handling by the nephrons is relatively well-preserved until the GFR falls to <10% of normal. Therefore, many patients with renal impairment can maintain a near normal, serum potassium concentration. They are still prone to developing hyperkalemia if excessive potassium is consumed and when renal function deteriorates.[9,12]

Increased potassium intake rarely causes any problem in subjects in the absence of significant renal impairment. With normal renal function, increased potassium intake will lead

TABLE 12-7. Signs, Symptoms, and Effects of Hypokalemia on Various Organ Systems

- Cardiovascular
  - Decrease in T-wave amplitude
  - Development of U waves
  - Hypotension
  - Increased risk of digoxin toxicity
  - PR prolongation (with severe hypokalemia)
  - Rhythm disturbances
  - ST segment depression
  - QRS widening (with severe hypokalemia)
- Metabolic/endocrine (mostly serve as compensatory mechanisms)
  - Decreased aldosterone release
  - Decreased insulin release
  - Decreased renal responsiveness to antidiuretic hormone
- Neuromuscular
  - Areflexia (with severe hypokalemia)
  - Cramps
  - Loss of smooth muscle function (ileus and urinary retention with severe hypokalemia)
  - Weakness
- Renal
  - Inability to concentrate urine
  - Nephropathy

TABLE 12-8. Etiologies of Hyperkalemia

- Extracellular shifting of potassium associated with acidemia
- True excess
  - Increased release of Intracellular potassium into blood stream
    - Hemolysis
    - Rhabdomyolysis
    - Muscle crush injuries
    - Burns
  - Increased total body potassium
    - Increased potassium intake (e.g., salt substitute, diet)
  - Decreased output or increased retention
  - Chronic or acute renal failure
  - Drugs
    - Potassium-sparing diuretics
    - Angiotensin-converting enzyme inhibitors
    - Nonsteroidal anti-inflammatory agents
    - Angiotensin II receptor antagonists
    - Unfractionated heparin
    - Trimethoprim (including drugs such as co-trimoxazole)
  - Deficiency of adrenal steroids
  - Addison disease

to increased renal excretion and redistribution to the intracellular space through the action of aldosterone and insulin, respectively. Interference with either mechanism may result in hyperkalemia. Decreased aldosterone secretion can occur with Addison disease or other defects affecting the hormone's adrenal output.[7,12] Pathological changes affecting the proximal or distal renal tubules also can lead to hyperkalemia.[7,12]

Use of potassium-sparing diuretics (e.g., spironolactone) is a common cause of hyperkalemia, especially in patients with renal function impairment. Concurrent use of potassium supplements (including potassium-rich salt substitutes) also will increase the risk. Similar to hypokalemia, hyperkalemia can result from transcellular shifting of potassium. In the presence of severe acidemia, potassium shifts from the intracellular to the extracellular space, which may result in a clinically significant increase in the serum potassium concentration.[10]

Clinical diagnosis. The cardiovascular manifestations of hyperkalemia are of major concern. They include cardiac rhythm disturbances, bradycardia, hypotension, and, in severe cases, cardiac arrest. At times, muscle weakness may occur before these cardiac signs and symptoms. To appreciate the potent effect of potassium on the heart, one has to realize that potassium is the principal component of cardioplegic solutions commonly used to arrest the rhythm of the heart during cardiac surgeries.[7,9,12]

Causes of spurious laboratory results. Several conditions will result in fictitious hyperkalemia in which the high serum concentration reported is not expected to have any significant clinical sequelae. Erythrocytes, similar to other cells, have high potassium content. When there is substantial hemolysis in the specimen collection tube, the red cells will release potassium in quantities large enough to produce misleading results. Hemolysis may occur when a very small needle is used for blood draw, the tourniquet is too tight, or when the specimen stands too long or is mishandled. When a high serum potassium concentration is reported in a patient without pertinent signs and symptoms, the test needs to be repeated to rule out hemolysis.[6,7,10]

A similar phenomenon can occur when the specimen is allowed to clot (when nonheparinized tubes are used) because platelets and white cells are also rich in potassium. In patients with leukemia or thrombocytosis, the potassium concentration should be obtained from plasma rather than serum samples. The normal plasma potassium concentration is 0.3–0.4 mEq/L lower than the serum values.

Management of chronic hyperkalemia includes decreasing dietary intake of potassium, discontinuing medications that increase serum potassium. For rapid correction of acute, symptomatic hyperkalemia, measures include: correcting metabolic acidosis with IV sodium bicarbonate; administering IV glucose and insulin or inhaled β-adrenergic agonists to shift potassium from the intravascular to the intracellular space; using high doses of loop diuretics to enhance renal excretion of potassium; administering sodium polystyrene sulfonate to increase colonic elimination of potassium; or initiating dialysis in the most severe cases. (**Minicase 3**.)

Chloride

Normal range: 95–103 mEq/L (95–103 mmol/L)

Physiology

Chloride is the most abundant extracellular anion with a low intracellular concentration (about 4 mEq/L). Chloride is passively absorbed from the upper small intestine. In the distal ileum and large intestine, its absorption is coupled with bicarbonate ion secretion. Chloride is primarily regulated by the renal proximal tubules, where it is exchanged for bicarbonate ions. Throughout the rest of the nephron, chloride passively follows sodium and water. In addition, the luminal and interstitial Cl/HCO_3 exchangers in the collecting duct also contribute to the renal regulation of chloride.

Chloride is influenced by the extracellular fluid balance and acid–base balance.[19,20] Although homeostatic mechanisms do not directly regulate chloride, they indirectly regulate it through changes in sodium and bicarbonate. The physiological role of chloride is primarily passive. It balances out positive charges in the extracellular fluid and, by passively following sodium, helps to maintain extracellular osmolality.

Hypochloremia and Hyperchloremia

Serum chloride values are used as confirmatory tests to identify fluid balance and acid–base abnormalities.[21] Like sodium, a change in the serum chloride concentration does not necessarily reflect a change in total body content. Rather, it indicates an alteration in fluid status and acid–base balance. One of the most common causes of hyperchloremia in hospitalized patients results from saline infusion. Chloride has the added feature of being influenced by bicarbonate. Therefore, it would be expected to decrease to the same proportion as sodium when serum is diluted with fluid and to increase to the same proportion as sodium during dehydration. However, when a patient is on acid-suppressive therapy (e.g., high-dose H2-blockers or proton pump inhibitors), has been receiving continuous or frequent nasogastric suction, or has profuse vomiting, a greater loss of chloride than sodium can occur because gastric fluid contains 1.5–3 times more chloride than sodium. Gastric outlet obstruction, protracted vomiting and self-induced vomiting also can lead to hypochloremia.

Drug and parenteral nutrition causes. Even though drugs can influence serum chloride concentrations, they rarely do so directly. For example, although loop diuretics (e.g., furosemide) and thiazide diuretics (e.g., hydrochlorothiazide) inhibit chloride uptake at the loop of Henle and distal nephron, respectively, the hypochloremia that may result is due to the concurrent loss of sodium and contraction alkalosis.[18,21] Because chloride passively follows sodium, salt and water retention can transiently raise serum chloride concentrations. This effect occurs with corticosteroids, and nonsteroidal anti-inflammatory agents (NSAIDs) such as ibuprofen. Also, parenteral nutrition solutions with high chloride concentrations are associated with an increased risk of hyperchloremia. Acetate or phosphate salts used in place of chloride salts (e.g., potassium chloride) reduce this risk. Acetazolamide also can cause hyperchloremia.

MINICASE 3

A Case of Hyperkalemia

Gary O., a 68-year-old man, is admitted to the cardiology service for further workup of dyspnea and shortness of breath. His chief complaints include worsening of shortness of breath in the last two days, swelling of his legs, and the need for extra pillows before he can go to bed for the past week. He experiences worsening fatigue and dyspnea with ordinary activities.

He admits to skipping his furosemide doses for the past two to three days because he does not like going to the bathroom all the time. He has been told that he needs furosemide for worsening shortness of breath. Otherwise, he takes his other medications "religiously."

Past medical history includes congestive heart failure (ejection fraction of 31% checked 5½ months ago), chronic atrial fibrillation, and type 2 diabetes mellitus.

Home medications: carvedilol 12.5 mg q 12 hr, furosemide 60 mg PO every morning and 20 mg every evening, glargine insulin 30 units daily, lisinopril 40 mg twice daily, potassium chloride 20 mEq PO daily, spironolactone 12.5 mg daily, and warfarin 5 mg daily.

Vital signs on admission: BP 110/78 mm Hg, HR 69 beats/min (baseline BP 118/82 mm Hg, and HR 68 beats/min); and weight: 80 kg (four weeks ago, clinic record), 90 kg (on admission).

Laboratory results: BNP: 532 pg/mL, sodium 133 mEq/L, potassium 5.7 mEq/L, chloride 101 mEq/L, CO_2 content 22 mEq/L, BUN 37 mg/dL, creatinine 2.1 mg/dL, (baseline creatinine 1.5 mg/dL), and glucose 72 mg/dL.

Other tests: EKG, atrial fibrillation, unchanged from baseline; oxygen saturation 92% on room air.

QUESTION: How would you interpret this patient's serum potassium concentration?

DISCUSSION: His serum potassium concentration, at 5.7 mEq/L is elevated. It is possible that his baseline potassium concentration is mildly elevated because there are several factors that would contribute to hyperkalemia: (1) he is taking a potassium supplement; (2) he is taking two drugs that can increase serum potassium—spironolactone and lisinopril; and (3) he has been renal insufficiency at baseline (creatinine 1.5 mg/dL). It is likely that his potassium concentration has increased more significantly in the last two days. His current state of hyperkalemia is likely exacerbated by two recent events: (1) nonadherence with furosemide in the last three days, which results in decreased renal potassium loss; and (2) worsening of heart failure (as suggested by increased BNP, weight gain of 10 kg, and increased leg swelling), which in turn decreases renal blood flow and results in worsening of acute renal failure (as suggested by an increased serum creatinine from 1.5–2.1 mg/dL)

The primary goal for managing hyperkalemia is to prevent/reverse cardiac symptoms. With a serum potassium of 5.7 mEq/L, there is a definite risk for arrhythmias. Therefore, a 12-lead EKG should be performed. If EKG changes are present and consistent with hyperkalemia, interventions that will decrease serum potassium concentration, such as IV insulin and dextrose or IV sodium bicarbonate, should be initiated right away. IV calcium (calcium gluconate 1 g) also should be administered to reduce the risk of arrhythmias. Regardless of the cardiac symptoms, his potassium supplement should be withheld. Because his blood pressure is not elevated, it also is reasonable to withhold spironolactone for now until the potassium concentration starts to decline.

In summary, this patient has hyperkalemia, most likely exacerbated by acute renal failure and continued use of a potassium supplement. Assessment of symptoms and signs of hyperkalemia should be performed as soon as possible.

Acid–base status and other causes. Acid–base balance is partly regulated by renal production and excretion of bicarbonate ions. The proximal tubules are the primary regulators of bicarbonate. These cells exchange bicarbonate with chloride to maintain the intracellular electropotential gradient. Renal excretion of chloride increases during metabolic alkalosis, resulting in a reduced serum chloride concentration.

The opposite situation also may be true: metabolic or respiratory acidosis results in an elevated serum chloride concentration. Hyperchloremic metabolic acidosis is not common but may occur when the kidneys are unable to conserve bicarbonate, as in interstitial renal disease (e.g., obstruction, pyelonephritis, and analgesic nephropathy), GI bicarbonate loss (e.g., cholera and staphylococcal infections of the intestines), and acetazolamide-induced carbonic anhydrase inhibition. Falsely elevated chloride is rare but may occur with bromide toxicity due to an inability to distinguish between these two halogens by the laboratory's chemical analyzer. Because the signs and symptoms associated with hyperchloremia and hypochloremia are related to fluid status or the patient's acid–base status and its underlying causes, rather than to chloride itself, the reader is referred to discussions in Chapter 13.

OTHER MINERALS

Magnesium

Normal range: 1.7–2.4 mg/dL (0.7–0.99 mmol/L) or 1.4–2 mEq/L

Physiology

Magnesium has a widespread physiological role in maintaining neuromuscular functions and enzymatic functions. Magnesium acts as a cofactor for phosphorylation of ATPs from adenosine phosphates. Magnesium also is vital for binding macromolecules to organelles (e.g., messenger ribonucleic acid to ribosomes).

The average adult body contains 21–28 g (1750–2400 mEq) of magnesium with the following distribution:

- About 50% in bone (about 30% or less of this pool is slowly exchangeable with extracellular fluid)
- 20% in muscle
- Around 10% in nonmuscle soft tissues
- 1–2% in extracellular fluid (for plasma magnesium, about 50% is free; approximately 15% is complexed to anions; and 30% is bound to protein, primarily albumin)

Approximately 30–40% of the ingested magnesium is absorbed from the jejunum and ileum through transcellular and paracellular mechanisms. Both passive diffusion down an electrochemical gradient and active transport process are involved. The extent of magnesium absorption may be affected by dietary magnesium intake, calcium intake, vitamin D, and PTH. However, conflicting data are available and the extent of these parameters in affecting absorption is unresolved. Certain medications (e.g., cyclosporine, tacrolimus, cisplatin, amphotericin B) can significantly increase renal magnesium loss, predisposing the patient to hypomagnesemia.

Urinary magnesium accounts for one third of the total daily magnesium output, while the other two thirds are in the GI tract (e.g., stool). Unbound serum magnesium is freely filtered at the glomerulus. All but about 3–5% of filtered magnesium is normally reabsorbed (100 mg/day). In other words, 97% of the filtered magnesium is reabsorbed under normal physiology. Reabsorption is primarily through the ascending limb of the loop of Henle (50–60%). About 30% is reabsorbed in the proximal tubule and 7% from the distal tubule. This explains why loop diuretics have a profound effect on renal magnesium wasting. The drive of magnesium reabsorption is mediated by the charge difference generated by the sodium-potassium-chloride cotransport system in the lumen.

The regulation of magnesium is primarily driven by the plasma magnesium concentration. Changes in plasma magnesium concentrations have potent effects on renal reabsorption and stool losses. These effects are seen over three to five days and may persist for a long time. Hormonal regulation of magnesium seems to be much less critical for its homeostasis.

Factors affecting calcium homeostasis also affect magnesium homeostasis.[22] A decline in serum magnesium concentration stimulates the release of PTH, which increases serum magnesium by increasing its release from the bone store and renal reabsorption. Hyperaldosteronism causes increased magnesium renal excretion. Insulin by itself does not alter the serum magnesium concentration. But in a hyperglycemic state, insulin causes rapid intracellular uptake of glucose. This process causes an increase in the phosphorylation by sodium–potassium ATPase on the cell membrane. Because magnesium is utilized as a cofactor for sodium potassium ATPase, serum magnesium concentration declines, resulting in hypomagnesemia. Excretion of magnesium is influenced by serum calcium and phosphate concentrations. Magnesium movement generally follows that of phosphate (i.e., if phosphate declines, magnesium also declines) and is the opposite of calcium.[21,22]

Other factors that increase magnesium reabsorption include acute metabolic acidosis, hyperthyroidism, and chronic alcohol use. Magnesium also regulates neuromuscular function. Magnesium depletion results in neuromuscular weakness as the release of acetylcholine to motor endplates is enhanced by the presence of magnesium. Motor endplate sensitivity to acetylcholine also is affected. When serum magnesium decreases, acetylcholine release increases, resulting in increased muscle excitation and this may lead to increased reflexes. Common symptoms associated with hypomagnesemia include weakness, muscle fasciculation with tremor, tetany, and increased reflexes. In addition, vasodilation may occur by a direct effect on blood vessels and ganglionic blockade due to hypomagnesemia.

Hypomagnesemia

Hypomagnesemia is defined as a serum magnesium concentration <1.7 mg/dL (<0.7 mmol/L). The common causes of hypomagnesemia include renal wasting, chronic alcohol use, diabetes mellitus, protein-calorie malnutrition, refeeding syndrome, and postparathyroidectomy. Because serum magnesium deficiency can be offset by magnesium release from bone, muscle, and the heart, the serum value may not be a useful indicator of cellular depletion and complications (e.g., arrhythmias). However, low serum magnesium usually indicates low cellular magnesium as long as the patient has a normal extracellular fluid volume.[22,23]

Causes. Magnesium deficiency is more common than magnesium excess. Depletion usually results from excessive loss from the GI tract or kidneys (e.g., use of loop diuretics). Magnesium depletion is not commonly the result of decreased intake because the kidneys can cease magnesium elimination in four to seven days to conserve the ion. However, with chronic alcohol consumption, deficiency can occur from a combination of poor intake, poor GI absorption (e.g., vomiting or diarrhea), and increased renal elimination. Depletion also can occur from poor intestinal absorption (e.g., small-bowel resection). Diarrhea can be a source of magnesium loss because diarrhea stools may contain as much as 14 mEq/L (7 mmol/L) of magnesium. Chronic use of proton-pump inhibitors also has been linked to hypomagnesemia.

Urinary magnesium loss may result from diuresis or tubular defects, such as the diuretic phase of acute tubular necrosis. Some patients with hypoparathyroidism may exhibit low magnesium serum concentrations from renal loss and, possibly, decreased intestinal absorption. Other conditions associated with magnesium deficiency include hyperthyroidism, primary aldosteronism, diabetic ketoacidosis, and pancreatitis. Magnesium deficiency associated with these conditions may be particularly dangerous because often there are concurrent potassium and calcium deficiencies. Although loop diuretics lead to significant magnesium depletion, thiazide diuretics do not cause hypomagnesemia, especially at lower doses (<50 mg/day). Furthermore, potassium-sparing diuretics (e.g., spironolactone, triamterene, and amiloride), are also magnesium-sparing and have some limited clinical role in diuretic-induced hypokalemia and hypomagnesemia.[22,24]

Clinical diagnosis. Magnesium also affects the central nervous system (CNS). Magnesium depletion can cause personality changes, disorientation, convulsions, psychosis, stupor, and coma.[22,25] Severe hypomagnesemia may result in hypocalcemia due to intracellular cationic shifts. Many symptoms of magnesium deficiency result from concurrent hypocalcemia.

Perhaps the most important effects of magnesium imbalance are on the heart. Decreased magnesium in cardiac cells may manifest as a prolonged QT interval, which is associated with an increased risk of arrhythmias, especially torsades de pointes.[25] Moderately decreased concentrations can cause electrocardiogram (EKG) abnormalities similar to those observed with hypokalemia.

A 24-hour urine magnesium excretion test may be helpful in determining the magnitude of a total body magnesium deficiency. If the value is normal, the patient is not considered deficient as long as serum magnesium is also normal. The diagnosis of total body magnesium deficiency is established when the 24-hour urinary magnesium excretion is low even in the presence of normal serum magnesium concentration.

Hypermagnesemia

Hypermagnesemia is defined as a serum magnesium concentration >2.4 mg/dL (>0.99 mmol/L).

Causes. Besides magnesium overload (e.g., overreplacement of magnesium, treatment for preeclampsia, and antacid/laxative overuse), the most important risk factor for hypermagnesemia is renal dysfunction. Rapid infusions of IV solutions containing large amounts of magnesium, such as those given for myocardial infarction, preeclampsia, and status asthmaticus, may result in hypermagnesemia.

Clinical diagnosis. Plasma magnesium concentrations below 6 mg/dL (<2.5 mmol/L) rarely cause serious symptoms. Nonspecific symptoms, such as muscle weakness, decrease in deep tendon reflexes or fatigue, may be present. As magnesium concentration rises above 6 mg/dL, more notable symptoms such as lethargy, mental confusion, and hypotension may be observed (**Table 12-9**).[22,24,27] In severe hypermagnesemia (12 mg/dL), life-threatening symptoms, including coma, paralysis, or cardiac arrest, can be observed and urgent therapy is indicated.

Treatment for severe or symptomatic hypermagnesemia may include IV calcium gluconate 1–2 g over 30 minutes to reverse the neuromuscular and cardiovascular blockade of magnesium. Increased renal elimination of magnesium can be achieved by forced diuresis with IV saline hydration and a loop diuretic agent. Hemodialysis should be reserved as a last resort.

TABLE 12-9. Signs and Symptoms of Hypermagnesemia

6–8.5 mg/dL	5–7 mEq/L	Bradycardia, flushing, sweating, sensation of warmth, fatigue, drowsiness
8.5–12 mg/dL	7–10 mEq/L	Lower blood pressure, decreased deep-tendon reflexes, altered mental status possible
>12 mg/dL	>10 mEq/L	Flaccid paralysis and increased PR and QRS intervals, severe mental confusion, coma, respiratory distress and asystole

Calcium

Normal range: 9.2–11 mg/dL (2.3–2.8 mmol/L) for adults

Physiology

Calcium plays an important role in the propagation of neuromuscular activity; regulation of endocrine functions (e.g., pancreatic insulin release and gastric hydrogen secretion), blood coagulation including platelet aggregation, and bone and tooth metabolism.[2,28]

The serum calcium concentration is closely regulated by complex interactions among PTH, serum phosphate, vitamin D system, and the target organ (**Figure 12-4**). About one third of the ingested calcium is actively absorbed from the proximal area of the small intestine, facilitated by 1,25-dihydroxycholecalciferol (1,25-DHCC or calcitriol, the most active form of vitamin D). Passive intestinal absorption is negligible with intake of <2 g/day. The average daily calcium intake is 2–2.5 g/day.

The normal adult body contains about 1000 g of calcium, with only 0.5% found in the extracellular fluid; 99.5% is integrated into bones. Therefore, the tissue concentration of calcium is small. Because bone is constantly remodeled by osteoblasts and osteoclasts, a small quantity of bone calcium is in equilibrium with the extracellular fluid. Extracellular calcium exists in three forms:

1. Complexed to bicarbonate, citrates, and phosphates (6%)
2. Protein bound, mostly to albumin (40%)
3. Ionized or free fraction (54%)

Intracellular calcium. Imbalance of body calcium results in disturbances in muscle contraction and nerve action.[28] Within the cells, calcium maintains a low concentration. The calcium that is attracted into the negatively charged cell is either actively pumped out or sequestered by mitochondria or the endoplasmic reticulum. Such differences in concentrations allow calcium to be used for transmembrane signaling. In response to stimuli, calcium is allowed either to enter a cell or released from internal cellular stores where it interacts with specific intracellular proteins to regulate cellular functions or metabolic processes.[2,26,27] Calcium enters cells through one of the three types of calcium channels that have been identified: T (transient or fast), N (neuronal), and L (long lasting or slow). Subsets of these channels may exist. Calcium channel-blockers are likely to affect the L channels.[29]

In muscle, calcium is released from the intracellular sarcoplasmic reticulum. The released calcium binds to troponin and stops troponin from inhibiting the interaction of actin and myosin. This interaction results in muscle contraction. Muscle relaxation occurs when calcium is pumped back into the sarcoplasmic reticulum. In cardiac tissue, calcium becomes important during phase 2 of the action potential. During this phase, fast entry of sodium stops and calcium entry through the slow channels begins (**Figure 12-5**), resulting in contraction. During repolarization, calcium is actively pumped out of the cell.[2]

Calcium channel-blocking drugs (e.g., nifedipine, diltiazem, and verapamil) inhibit the movement of calcium into muscle cells, thus decreasing the strength of contraction. The areas

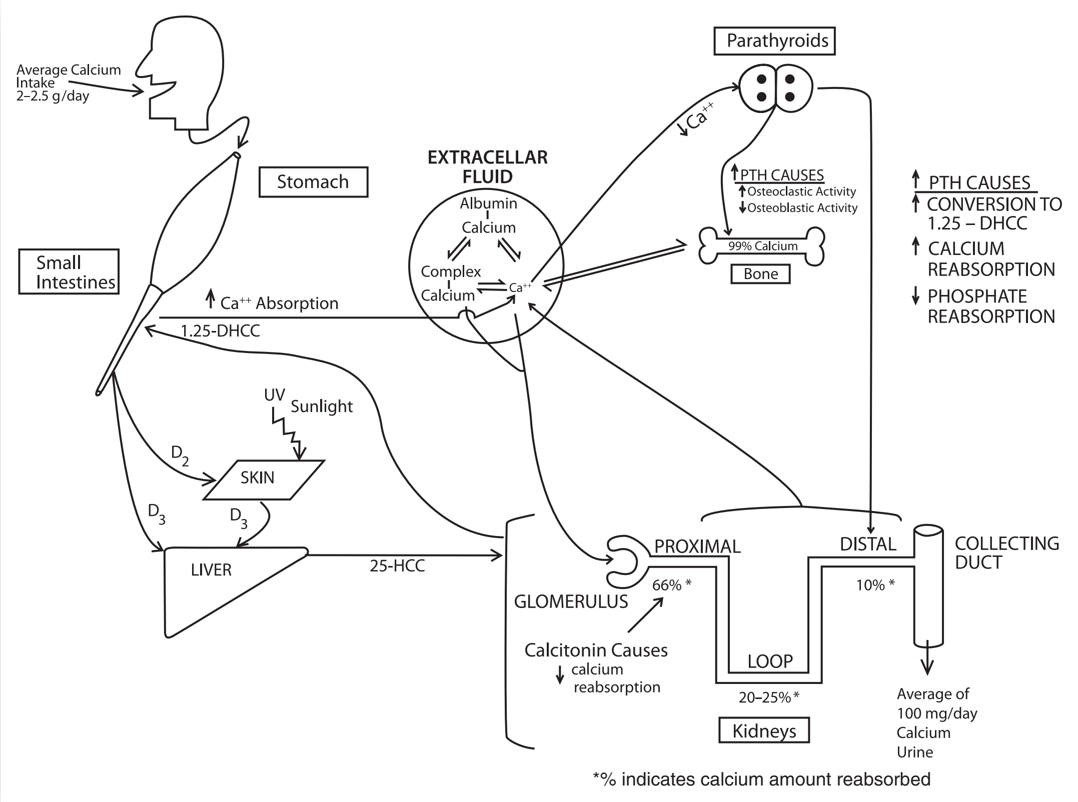
Average Calcium Intake 2–2.5 g/day
Stomach
Small Intestines
↑ Ca++ Absorption
1.25-DHCC
UV Sunlight
D2
SKIN
D3
D3
LIVER
25-HCC
EXTRACELLAR FLUID
Albumin
Calcium
Complex
Calcium
Ca++
Parathyroids
↓Ca++
↑PTH CAUSES
↑Osteoclastic Activity
↓Osteoblastic Activity
99% Calcium
Bone
↑ PTH CAUSES
↑ CONVERSION TO 1.25 – DHCC
↑ CALCIUM REABSORPTION
↓ PHOSPHATE REABSORPTION
PROXIMAL
DISTAL
COLLECTING DUCT
66% *
10% *
GLOMERULUS
Calcitonin Causes ↓ calcium reabsorption
LOOP
20–25%*
Kidneys
Average of 100 mg/day Calcium Urine
*% indicates calcium amount reabsorbed

FIGURE 12-4. Calcium physiology: relationship with vitamin D, calcitonin, PTH, and albumin. The primary source of calcium is from diet. Absorption of calcium takes place in the small intestine. Vitamin D, more specifically, calcitriol or 1,25-DHCC, has the most potent effect on intestinal extraction of calcium. Once absorbed, calcium is transported in the extracellular fluid by albumin to various organs. Bones serve as an important reservoir for calcium. When serum calcium concentration decreases, PTH release is increased and it stimulates osteoclast activity, which releases calcium into the plasma to maintain normocalcemia. Calcium also is excreted renally. Only about 10% of dietary calcium is normally lost in the urine.

Although humans can synthesize a limited amount of vitamin D with optimal UVB exposure, the majority of vitamin D comes from the diet, which many include ergocalciferol (vitamin D_2, primarily from plants) and cholecalciferol (vitamin D_3, primarily from animal sources). The endogenous vitamin D formed by the body is cholecalciferol (D_3).

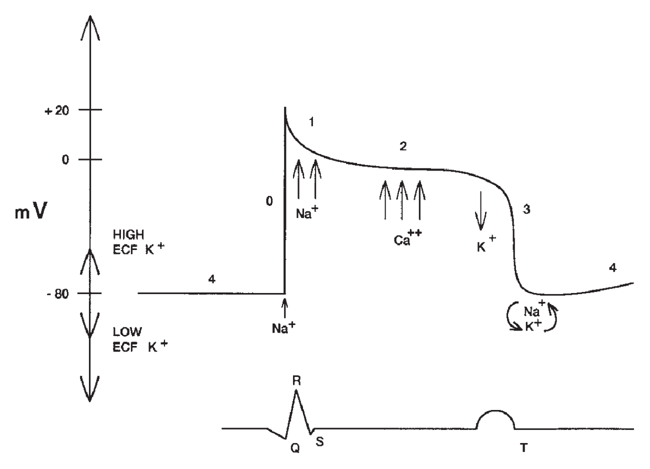

FIGURE 12-5. Cardiac intracellular potential and its relationship to the EKG.

that are most sensitive to these effects appear to be the sinoatrial and atrioventricular nodes and vascular smooth muscles, which explains the hypotensive effects of nifedipine.

Extracellular calcium. Complexed calcium usually accounts for <1 mg/dL (<0.25 mmol/L) of blood calcium. The complex usually is formed with bicarbonate, citrate, or phosphate. In patients with CKD, calcium also may be bound with sulfate because the anion is retained. Phosphate plays an important role in calcium homeostasis. Under normal physiological conditions, the product of calcium concentration and phosphate concentration (calcium–phosphate product) is relatively constant: an increase in one ion necessitates a corresponding decline in the other. In addition, many homeostatic mechanisms that control calcium also regulate phosphate. This relationship is particularly important in renal failure; the decreased phosphate excretion may ultimately lead, through a complex mechanism, to hypocalcemia, especially if the hyperphosphatemia is untreated.[30,31]

Protein-bound calcium is bound primarily to serum albumin (80%) and globulins (20%). Protein-bound calcium is in equilibrium with ionized calcium, which is affected by the serum anion concentration and blood pH. This equilibrium is important because ionized calcium is the physiologically active moiety. Alkalosis increases protein binding of calcium, resulting in a lower free fraction, whereas acidosis has the opposite effect. In patients with respiratory or metabolic alkalosis, the signs and symptoms of hypocalcemia may become more pronounced due to increased binding. Conversely, signs and symptoms of hypercalcemia become more apparent in patients with metabolic or respiratory acidosis. Therefore, total serum calcium concentration, which is commonly reported by clinical laboratories, is not as clinically significant as the quantity of available ionized calcium. In fact, it is the free calcium concentration that is closely regulated by the different homeostatic mechanisms.

Clinically, serum protein concentrations, especially albumin, have an important influence in regulating the amount of physiologically active calcium in the serum. The normal serum calcium range is 9.2–11 mg/dL (2.3–2.8 mmol/L) for a patient with a serum albumin of approximately 4 g/dL. In normal healthy adults, only 40–50% of the total serum calcium is free from protein-binding and thus considered as physiologically active. In patients with hypoalbuminemia (due to acute illnesses, severe malnutrition), the free concentration of calcium is elevated despite a “normal” total serum calcium concentration. Therefore, it is a common practice to either measure ionized calcium or to correct the total serum calcium concentration based on the measured albumin concentration. The following formula is commonly used in an attempt to “correct” total serum calcium concentration:

$$\text{Ca}_{\text{corr}} = ([4.0 - \text{albumin}] \times 0.8 \text{ mg/dL}) + \text{Ca}_{\text{uncorr}}$$

where Ca_{corr} is the corrected serum calcium concentration, and $\text{Ca}_{\text{uncorr}}$ is the uncorrected (or measured total) serum calcium concentration. For example, a clinician may be asked to write parenteral nutrition orders for an emaciated cancer patient. The serum albumin is 1.9 g/dL (19 g/L), and the total serum calcium concentration is 7.7 mg/dL (1.9 mmol/L). At first glance, one might consider the calcium to be low. But with the reduced serum albumin concentration, more ionized calcium is available to cells.

$$\text{Ca}_{\text{corr}} = ([4.0 - 1.9] \times 0.8) + 7.7 = 9.4 \text{ mg/dL (2.34 mmol/L)}$$

The corrected serum calcium concentration is, thus, within the normal range. More importantly, the patient does not exhibit any signs and symptoms of hypocalcemia. Calcium supplementation is not indicated. In the presence of severe hypoalbuminemia, as in critically ill patients, an apparently low total serum calcium may in fact be sufficient or in some instances, excessive. Administration of IV albumin can lead to a significant decline in serum calcium concentration due to the resultant increased binding. The measured total calcium concentration will need to be corrected with the new albumin concentration.

Although this serum calcium correction method may be useful, the clinician must be aware of its limitations and potential for inaccuracy. The correction factor of 0.8 represents an average fraction of calcium bound to albumin under normal physiology. To have an accurate determination of the free concentration, a direct measurement of serum ionized calcium concentration should be available in most clinical laboratories (normal range: 4–4.8 mg/dL or 1–1.2 mmol/L). Ultimately, the patient’s clinical presentation is the most important factor to determine if immediate treatment for a calcium disorder is indicated.

Although calcium absorption takes place throughout the entire small intestine, the proximal region of the small intestine (jejunum and proximal ileum) are the most active and regulated areas. Calcium absorption from the human GI tract is mediated by two processes: (1) transcellular active transport, a saturable, vitamin D–responsive process mediated by specific calcium binding proteins primarily in the upper GI tract, particularly in the distal duodenum and upper jejunum; and (2) paracellular processes, a nonsaturable linear transfer via diffusion that occurs throughout the entire length of the intestine. Under normal physiology, the total calcium absorptive capacity is the highest in the ileum because of the longer residence time. The rate of paracellular calcium absorption is fairly stable regardless of calcium intake. However, when dietary calcium intake is relatively limited, the efficiency of transcellular calcium transport becomes higher and accounts for a significant fraction of the absorbed calcium. Transcellular calcium transport is closely regulated by vitamin D, although other mechanisms also may be involved. Specifically, 1,25-DHCC induces the intestinal expressions of transcellular calcium transporters through its binding with the vitamin D receptors (VDR) in the intestinal epithelial cells.

Effect of vitamin D. A small amount of calcium is excreted daily into the GI tract through saliva, bile, and pancreatic and intestinal secretions. However, the primary route of elimination is filtration by the kidneys. Calcium is freely filtered at the glomeruli, where approximately 65% is reabsorbed at the proximal tubules under partial control by calcitonin and 1,25-DHCC. Roughly 25% is reabsorbed in the loop of Henle, and another 10% is reabsorbed at the distal tubules under the influence of PTH.

Despite being classified as a vitamin, the physiological functions of vitamin D more closely resemble a hormone. Vitamin D is important for the following:

- Intestinal absorption of calcium
- PTH-induced mobilization of calcium from bone
- Calcium reabsorption in the proximal renal tubules
- Vitamin D must undergo several conversion steps before the active form, calcitriol or 1,25-DHCC, is formed. It is absorbed by the intestines in two forms, 7-dehydrocholesterol and cholecalciferol. 7-dehydrocholesterol is converted into cholecalciferol in the skin by the sun’s ultraviolet radiation. Hepatic and intestinal enzymes, including CYP27A1, CYP2J2 and CYP3A4, convert cholecalciferol to 25-hydroxycholecalciferol

(25-HCC or calcidiol or calcifediol), which is then further activated by CYP27B1 in the kidneys to form the active 1,25-DHCC or calcitriol. This last conversion step is regulated by PTH. When PTH is increased during hypocalcemia, renal production of calcitriol increases, which increases intestinal absorption of calcium.[30,32]

Influence of calcitonin. Calcitonin is a hormone secreted by specialized C cells of the thyroid gland in response to a high level of circulating ionized calcium. Calcitonin lowers serum calcium levels in part by inhibiting osteoclastic activity, thereby inhibiting bone resorption. Also, it decreases calcium reabsorption in the renal proximal tubules to result in increased renal calcium clearance.[28] Calcitonin is used for the treatment of acute hypercalcemia and several different forms of the hormone are available.

Influence of parathyroid hormone. PTH is the most important hormone involved in calcium homeostasis. It is secreted by the parathyroid glands, which are embedded in the thyroid, in direct response to low circulating ionized calcium. PTH closely regulates, and also is regulated by the vitamin D system to maintain the serum ionized calcium concentration within a narrow range. Generally, PTH increases the serum calcium concentration and stimulates the enzymatic activity of CYP27B1 to promote renal conversion of calcidiol to calcitriol, which enhances intestinal calcium absorption. Conversely, calcitriol is a potent suppressor of PTH synthesis via a direct mechanism that is independent of the serum calcium concentration.[28,31] The normal reference range for serum PTH concentrations is 10–65 pg/mL (10–65 ng/L).

Tubular reabsorption of calcium and phosphate at the distal nephron is controlled by PTH; it increases renal reabsorption of calcium and decreases the reabsorption of phosphate, resulting in lower serum phosphate and higher serum calcium concentrations. Perhaps the most important effect of PTH is on the bone. In the presence of PTH, osteoblastic activity is diminished and bone resorption processes of osteoclasts are increased. These effects increase serum ionized calcium, which feeds back to the parathyroid glands to decrease PTH output.[30]

The suppressive effect of calcitriol on PTH secretion is used clinically in patients with CKD who have excessively high serum PTH concentrations due to secondary hyperparathyroidism. PTH is a known uremic toxin, and its presence in supraphysiological concentrations has many adverse effects (e.g., suppression of bone marrow erythropoiesis and increased osteoclastic bone resorption with replacement by fibrous tissue).[33] **Figure 12-6** depicts the relationship between serum PTH and serum calcium concentrations.

Abnormalities. True abnormal serum concentrations of calcium may result from an abnormality in any of the previously mentioned mechanisms, including the following:

- Altered intestinal absorption[8,30,31,34]
- Altered number or activity of osteoclast and osteoblast cells in bone[8,30,31,34]
- Changes in renal reabsorption of calcium[8,30,31,34]
- Calcium or phosphate IV infusions

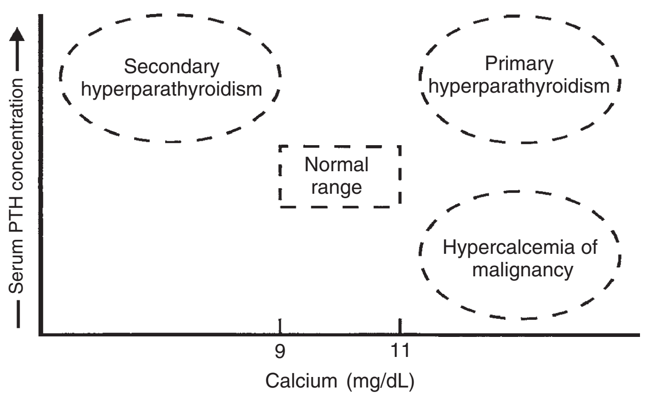
Secondary hyperparathyroidism
Primary hyperparathyroidism
Normal range
Hypercalcemia of malignancy
Serum PTH concentration
9
11
Calcium (mg/dL)

FIGURE 12-6. Interpretation of serum PTH concentrations with concomitant serum calcium concentrations.

Patients with CKD have increased serum phosphate and decreased serum calcium concentrations as a result of the following factors that interact via a complex mechanism: decreased phosphate clearance by the kidneys, decreased renal production of calcitriol, and skeletal resistance to the calcemic action of PTH. This interaction is further complicated by the metabolic acidosis of renal failure, which can increase bone resorption to result in decreased bone integrity.

Hypocalcemia

Hypocalcemia indicates a total serum calcium concentration of <9.2 mg/dL (<2.3 mmol/L). The most common cause of hypocalcemia is low serum proteins. As discussed previously, decreased serum protein leads to an increased free fraction of ionized calcium. If there is no other coexisting factor that could impair or alter calcium homeostasis, this should not be associated with a functional calcium deficit and clinical symptoms. Therefore, a serum protein concentration should always be taken into consideration when interpreting serum total calcium concentration. Even in the case of true, mild hypocalcemia, the patient may remain asymptomatic and often no treatment is required.

The most common causes of a true reduction in total serum calcium are disorders of vitamin D metabolism or impaired PTH production (**Table 12-10**). Osteomalacia (in adults) and

TABLE 12-10. Common Etiologies of Hypocalcemia
Diminished intake
Medications (see text)
Hyperphosphatemia
Hypoalbuminemia
Hypomagnesemia
Hypoparathyroidism (common)
Pancreatitis
Renal failure
Secondary hyperparathyroidism
Hypovitaminosis D (common)

rickets (in children) can result from severe deficiency in dietary calcium or vitamin D, diminished synthesis of vitamin D_3 from insufficient sunlight exposure, or resistance of the intestinal wall to the action of vitamin D. The reduction in serum calcium leads to secondary hyperparathyroidism, which increases bone resorption. Over a long period of time, bones lose their structural integrity and become more susceptible to fracture. The diminished serum calcium concentration, if significant, may result in tetany. Other notable findings may include EKG changes (QT prolongation) and arrhythmias.

Diminished intake. Although uncommon, diminished intake of calcium is an important cause of hypocalcemia, especially in patients receiving long-term total parental nutrition solutions.[37,38]

Medications. Excessive use of certain drugs to lower serum calcium by either increasing bone deposition or decreasing renal reabsorption of calcium may lead to hypocalcemia. These drugs include calcitonin, glucocorticoids, loop diuretics, etidronate, pamidronate, alendronate, cinacalcet, and denosumab.

IV bicarbonate administration and hyperventilation can lead to alkalemia, resulting in decreased ionized serum calcium. This decrease is usually important only in patients who already have low serum calcium concentrations. Other drugs that may cause hypocalcemia include phenytoin, phenobarbital, aluminum-containing antacids, and cisplatin.

Another cause is rapid IV administration of phosphate salts, especially at high doses. Phosphate can bind calcium and form an insoluble complex that can deposit into soft tissues and clog the microcirculation, causing metastatic calcification, hardening of normally pliable tissues, or blockage of capillary blood flow.[37,38] Soft-tissue deposition of the calcium–phosphate complex in lungs and blood vessels occurs when the serum solubility product of calcium times phosphate is high. The product of serum calcium and phosphate concentrations (both expressed in mg/dL) is often calculated, especially in patients with CKD, to minimize the risk for tissue calcification. An increased risk of deposition is likely in patients with a calcium–phosphate product that exceeds 50 or in patients with alkalosis. Other than the IV route, a large amount of phosphate may be absorbed from the GI tract with the use of certain enema and laxative preparations.[33]

Hypoparathyroidism. Hypoparathyroidism can reduce serum calcium concentrations. The most common cause of hypoparathyroidism is thyroidectomy, when the parathyroid glands are removed along with the thyroid glands. Because PTH is the major hormone regulating calcium balance, its absence significantly reduces serum calcium.[38]

Secondary hyperparathyroidism. Hypocalcemia is commonly seen in patients with secondary hyperparathyroidism resulting from CKD (Figure 12-6). The mechanism is complex and involves elevated serum phosphate concentrations and reduced activation of vitamin D. PTH acts on bone to increase calcium and phosphate resorption. Because renal phosphate elimination is reduced because of renal failure, the serum phosphate concentration is often high and depresses the serum calcium level. Because of the high phosphate concentrations in the intestinal lumen, dietary calcium is bound and absorption is impaired, while phosphate absorption continues.

Metabolic acidosis. Common in CKD, metabolic acidosis further enhances bone resorption. With prolonged severe hyperparathyroidism, excessive osteoclastic resorption of bones results in replacement of bone material with fibrous tissues. This condition is termed *osteitis fibrosa cystica.*[31,34,35] Such diminution of bone density may result in pathological fractures. Although the serum total calcium concentrations are low, patients may not show symptoms of hypocalcemia because the accompanying acidosis helps to maintain ionized serum calcium through the reduction in protein binding.

Magnesium. Similar to potassium, calcium balance is affected by magnesium homeostasis. Therefore, if a patient develops concurrent hypocalcemia and hypomagnesemia as a result of loop diuretic therapy, calcium replacement therapy may not be effective until magnesium balance is restored.

Clinical diagnosis. As with any electrolyte disorder, the severity of the clinical manifestations of hypocalcemia depends on the acuteness of onset. Hypocalcemia can at times be a medical emergency with symptoms primarily in the neuromuscular system.[37,38] They include fatigue, depression, memory loss, hallucinations, and, in severe cases, seizures, and tetany. The early signs of hypocalcemia are finger numbness, tingling and burning of extremities, and paresthesia. Mental instability and confusion may be seen in some patients as the primary manifestation.

Tetany is the hallmark of severe hypocalcemia. The mechanism of muscle fasciculation during tetany is the loss of the inhibitory effect of ionized calcium on muscle proteins. In extreme cases, this loss leads to increased neuromuscular excitability that can progress to laryngospasm and tonic-clonic seizures. Chvostek and Trousseau signs are hallmarks of hypocalcemia. The *Chvostek sign* is a unilateral spasm induced by a slight tap over the facial nerve. The *Trousseau sign* is a carpal spasm elicited when the upper arm is compressed by an inflated BP cuff.[31,38,40]

As hypocalcemia worsens, the cardiovascular system may be affected, as evidenced by myocardial failure, cardiac arrhythmias, and hypotension.[37,38] Special attention should be paid to serum calcium concentrations in patients receiving diuretics, corticosteroids, digoxin, antacids, lithium, and parenteral nutrition and in patients with renal disease.

Hypercalcemia

Hypercalcemia indicates a total serum calcium concentration >11 mg/dL (>2.8 mmol/L).

Causes. The most common causes of hypercalcemia are malignancy and primary hyperparathyroidism (Figure 12-6). Malignancies can increase serum calcium by several mechanisms. Osteolytic metastases can arise from breast, lung, thyroid, kidney, or bladder cancer. These tumor cells invade bone and produce substances that directly dissolve bone matrix and mineral content. Some malignancies, such as multiple myeloma, can produce factors that stimulate osteoclast proliferation and activity. Another mechanism is the ectopic production of PTH or PTH-like substances by tumor cells, resulting in a pseudohyperparathyroid state.[39,41]

In primary hyperparathyroidism, inappropriate secretion of PTH from the parathyroid gland, usually due to an adenoma, increases serum calcium concentrations. The other major cause of hypercalcemia in hyperparathyroidism is the increased renal conversion of calcidiol to calcitriol. As the serum calcium concentration rises, the renal ability to reabsorb calcium may be exceeded, leading to an increased urinary calcium concentration and the subsequent formation of calcium–phosphate and calcium–oxalate renal stones. Typically, this condition results from parathyroid adenomas but also may be caused by primary parathyroid hyperplasia of chief cells or parathyroid carcinomas.[31,41]

Approximately 2% of patients treated with thiazide diuretics may develop hypercalcemia. Patients at risk are those with hyperparathyroidism. The mechanism appears to be multifactorial and includes enhanced renal reabsorption of calcium and decreased plasma volume.

The milk-alkali syndrome (Burnett syndrome), rarely observed today, is another drug-related cause of hypercalcemia.[33] This syndrome occurs from a chronic high intake of milk or calcium products combined with an absorbable antacid (e.g., calcium carbonate, sodium bicarbonate, or magnesium hydroxide). This syndrome was more common in the past when milk or cream was used to treat gastric ulcers and before the advent of nonabsorbable antacids. Renal failure can occur as a result of calcium deposition in soft tissues.[33,42]

Hypercalcemia also can result from the following[28,40,41,43]:

- Excessive administration of IV calcium salts
- Calcium supplements
- Chronic immobilization
- Paget disease
- Sarcoidosis
- Hyperthyroidism
- Acute adrenal insufficiency
- Lithium-induced renal calcium reabsorption
- Excessive vitamin D, vitamin A, or thyroid hormone, which increases intestinal absorption
- Drugs (e.g., tamoxifen, teriparatide, androgenic hormones)

Clinical diagnosis. Similar to hypocalcemia and other electrolyte disorders, the severity of the clinical manifestations of hypercalcemia depends on the acuteness of onset. Hypercalcemia can be a medical emergency, especially when serum concentrations rise above 14 mg/dL (>3.5 mmol/L). Symptoms associated with this condition often consist of vague GI complaints such as nausea, vomiting, abdominal pain, dyspepsia, and anorexia. More severe GI complications include peptic ulcer disease, possibly due to increased gastrin release, and acute pancreatitis.[43,44]

Severe hypercalcemic symptoms primarily involve the neuromuscular system (e.g., lethargy, obtundation, psychosis, cerebellar ataxia, and, in severe cases, coma and death). However, EKG changes and spontaneous ventricular arrhythmias also may be seen. Also, it may enhance the inotropic effects of digoxin, increasing the likelihood of cardiac arrhythmias.[35-38]

Renal function may be affected by hypercalcemia through the ability of calcium to inhibit the adenyl cyclase–cyclic adenosine monophosphate system that mediates the ADH effects on the collecting ducts. This inhibition results in diminished conservation of water by the kidneys. The renal effect is further compounded by diminished solute transport in the loop of Henle, leading to polyuria, nocturia, and polydipsia.[28] Other chronic renal manifestations include nephrolithiasis, nephrocalcinosis, chronic interstitial nephritis, and renal tubular acidosis.

In addition, hypercalcemia can cause vasoconstriction of the renal vasculature, resulting in a decrease in renal blood flow and GFR. If hypercalcemia is allowed to progress, oliguric acute renal failure may ensue.[28] In the presence of high calcium–phosphate product, soft-tissue calcification by the calcium–phosphate complex may occur. The signs and symptoms described above are mostly seen in patients with severe hypercalcemia. With serum concentrations <13 mg/dL (3.2 mmol/L), most patients should be asymptomatic.

Causes of spurious laboratory results. False hypercalcemia can occur if the tourniquet is left in place too long when the blood specimen is drawn. This results from increased plasma-protein pooling in the phlebotomized arm. Falsely elevated calcium should be suspected if serum albumin is >5 g/dL (>50 g/L). **Table 12-11** contains the normal range values for tests related to calcium metabolism.

Phosphate

Normal range: 2.3–4.7 mg/dL (0.74–1.52 mmol/L) for adults

Many of the factors that influence serum calcium concentrations also affect serum phosphate, either directly or indirectly. Laboratory values for calcium and phosphate should, therefore, be interpreted together. Because phosphate exists as several organic and inorganic moieties in the body, some clinical laboratories simply report the phosphate value as phosphorus.

Physiology

Phosphate is a major intracellular anion with several functions. It is important for intracellular metabolism of proteins, lipids, and carbohydrates, and it is a major component

TABLE 12-11. Normal Ranges for Tests Related to Calcium Metabolism in Adults

Calcium (free)	4.6–5.8 mg/dL
Calcium (total)	9.2–11.0 mg/dL
Phosphate	2.3–4.7 mg/dL
PTH	10–65 pg/mL
Urine calcium	<250 mg/day in men <200 mg/day in women
Urine phosphate	1 g/day (average)

PTH = parathyroid hormone.

in phospholipid membranes, ribonucleic acids, nicotinamide diphosphate (an enzyme cofactor), cyclic adenine and guanine nucleotides (second messengers), and phosphoproteins. Another important function of phosphate is in the formation of high-energy bonds for the production of ATP, which is a source of energy for many cellular reactions. Phosphate is a component of 2,3-diphosphoglycerate (2,3-DPG), which regulates the release of oxygen from Hgb to tissues. In addition, phosphate has a regulatory role in the glycolysis and hydroxylation of cholecalciferol. It is also an important acid–base buffer.[35,40]

A balanced diet for adults usually contains about 800–1500 mg/day of phosphate. About two thirds is actively absorbed from the small intestine. Some of the phosphate is absorbed passively with calcium and some is absorbed under the influence of calcitriol, which also increases the intestinal absorption of calcium. However, phosphate is the first of the two to be absorbed.[40]

Phosphate absorption is diminished when a large amount of calcium or aluminum is present in the intestine due to the formation of insoluble phosphate compounds. Such large amounts of calcium and aluminum may result from the consumption of antacids. In fact, for patients with CKD who have high serum phosphate concentrations, calcium- and aluminum-containing antacids may be given with meals as phosphate binders to reduce intestinal phosphate absorption.[45] It should be noted that due to concerns of detrimental accumulation of aluminum in the CNS as well as the ability to worsen anemia and bone disease, chronic use of aluminum-containing antacids should be avoided.

Phosphate is widely distributed in the body throughout the plasma, extracellular fluid, cell membrane structures, intracellular fluid, collagen, and bone. Bone contains 85% of the phosphate in the body. About 90% of plasma phosphate is filtered at the glomeruli, and the majority is actively reabsorbed at the proximal tubule. Some reabsorption also takes place in the loop of Henle, distal tubules, and possibly the collecting ducts.[31] The amount of renal phosphate excretion is, therefore, the amount filtered minus the amount reabsorbed. Increased urinary phosphate excretion can result from an increase in plasma volume and the action of PTH, which can block phosphate reabsorption throughout the nephron. In contrast, vitamin D_3 and its metabolites can directly stimulate proximal tubular phosphate reabsorption. In all, 90% of eliminated phosphate is excreted renally, while the remainder is secreted into the intestine.[31,35,46] Renal handling of phosphate, especially the proximal tubules, therefore, plays an important role in maintaining the homeostatic balance of phosphate. Renal phosphate transport is active, saturable, and dependent on pH and sodium ion. However, fluctuation in serum phosphate mostly results from changes in either the GFR or the rate of tubular reabsorption.[2,31,35]

Serum phosphate and calcium concentrations as well as PTH and vitamin D levels are intimately related with each other. Serum phosphate indirectly controls PTH secretion via a negative feedback mechanism. With a decrease in the serum phosphate concentration, the conversion of calcidiol to calcitriol increases (which increases serum concentrations of both phosphate and calcium). Both the intestinal absorption and renal reabsorption of phosphate are increased. The concomitant increase in serum calcium then directly decreases PTH secretion. This decrease in serum PTH concentration permits a further increase in renal phosphate reabsorption.[30,31]

A true phosphate imbalance may result from an abnormality in any of the previously discussed mechanisms and hormones for maintaining calcium and phosphate homeostasis. They may include altered intestinal absorption, altered number or activity of osteoclast and osteoblast cells in bone, changes in renal calcium and phosphate reabsorption, and IV infusions of calcium or phosphate salts.[35,40]

Hypophosphatemia

Hypophosphatemia indicates a serum phosphate concentration of <2.3 mg/dL (<0.74 mmol/L). The following three mechanisms commonly contribute to decreased serum phosphate concentrations:

- Increased renal excretion[40,47,48]
- Intracellular shifting
- Decreased phosphate or vitamin D intake[41,47,48]

To identify the etiology of hypophosphatemia, the serum and urine phosphate concentrations should be evaluated simultaneously. Low urine and serum phosphates indicate either a diminished phosphate intake or excessive use of phosphate-binders. An increased urine phosphate suggests either hyperparathyroidism or renal tubular dysfunction. If the increased urine phosphate is accompanied by elevated serum calcium, the presence of primary hyperparathyroidism or decreased vitamin D metabolism must be considered.

Common causes. Hypophosphatemia commonly results from decreased renal reabsorption or increased GFR, a shift of phosphate from extracellular to intracellular fluid, alcoholism, or malnutrition. Phosphate is added to total parenteral nutrition solutions for muscle growth and replenishment of hepatic glycogen storage in malnourished patients. The infusion of concentrated glucose solution increases insulin secretion from the pancreas, which facilitates glucose and phosphate cell entry. Phosphate is used to form phosphorylated hexose intermediates during cellular utilization of glucose. An inadequate phosphate content in these nutritional fluids can decrease anabolism, glycolysis, and ATP and 2,3-DPG production.[48]

Infusion of concentrated glucose solutions, especially when accompanied by insulin, can produce hypophosphatemia through intracellular phosphate shifting. This condition, known as *refeeding syndrome*, can occur when an inadequate amount of phosphate is given during total parenteral nutrition (i.e., when a large amount of phosphate is taken up by the newly produced cells during anabolism).

Hypophosphatemia also can occur during treatment of hyperkalemia with insulin and dextrose. In addition, aluminum- and calcium-containing antacids, as well as magnesium hydroxide, are potent binders of intestinal phosphate.[45]

Overuse of these agents can severely reduce serum phosphate concentrations in patients with normal renal function. Moreover, calcitonin, glucagon, and β-adrenergic stimulants can decrease serum phosphate concentrations. Thiazide and loop diuretics can increase renal phosphate excretion. However, this effect is often insignificant clinically in otherwise healthy individuals.

Other conditions known to cause hypophosphatemia include nutritional recovery after starvation, treatment of diabetic ketoacidosis, decreased absorption or increased intestinal loss, alcohol withdrawal, the diuretic phase of acute tubular necrosis, and prolonged respiratory alkalosis. To compensate for respiratory alkalosis, carbon dioxide shifts from intracellular to extracellular fluid. This shift increases the intracellular fluid pH, which activates glycolysis and intracellular phosphate trapping. Metabolic acidosis, in contrast, produces a minimal change in serum phosphate.

Uncommon causes. Burn patients often retain a great amount of sodium and water. During wound healing, diuresis often ensues, which results in a substantial loss of phosphate. Because anabolism also occurs during recovery, hypophosphatemia may be inevitable without proper replacement. A moderate reduction in serum phosphate can occur from prolonged nasogastric suctioning, gastrectomy, small bowel or pancreatic disease resulting in malabsorption, and impaired renal phosphate reabsorption in patients with multiple myeloma, Fanconi syndrome, heavy-metal poisoning, amyloidosis, and nephrotic syndrome.[47,48]

Severe hypophosphatemia. Severe phosphate depletion (<1 mg/dL or <0.32 mmol/L) can occur during diabetic ketoacidosis. The resultant acidosis mobilizes bone, promotes intracellular organic substrate metabolism, and releases phosphate into the extracellular fluid. The glycosuria and ketonuria caused by diabetic ketoacidosis results in an osmotic diuresis that increases urinary phosphate excretion. The combined effects of these events may produce a normal serum phosphate concentration with severe intracellular deficiency. When diabetic ketoacidosis is corrected with insulin, phosphate accompanies glucose to move intracellularly. Serum phosphate is usually reduced within 24 hours of treatment. As the acidosis is corrected, there is further intracellular shifting of phosphate to result in profound hypophosphatemia. The accompanying volume repletion may exacerbate the hypophosphatemia further.

Clinical diagnosis. Patients with a moderate reduction in serum phosphate (2–2.3 mg/dL or 0.64–0.74 mmol/L) are often asymptomatic. Neurological irritability may occur as the serum phosphate concentration drops below 2 mg/dL (<0.64 mmol/L). Severe hypophosphatemia is often associated with muscle weakness, rhabdomyolysis, paresthesia, hemolysis, platelet dysfunction, and cardiac and respiratory failure.

CNS effects include encephalopathy, confusion, obtundation, seizures, and ultimately, coma. The mechanism for these effects may involve decreased glucose utilization by the brain, decreased brain cell ATP, or cerebral hypoxia from increased oxygen-Hgb affinity, secondary to diminished erythrocyte 2,3-DPG content. This decreased content results in decreased glycolysis, which leads to decreased 2,3-DPG and ATP production. The decreased contents of 2,3-DPG and ATP result in an increased affinity of Hgb for oxygen, eventually leading to decreased tissue oxygenation. The ensuing cerebral hypoxia may explain the persistent coma often seen in patients with diabetic ketoacidosis. Hemolysis may occur, but it is rarely seen at serum phosphate concentrations >0.5 mg/dL (>0.16 mmol/L).

Hyperphosphatemia

Hyperphosphatemia indicates a serum phosphate concentration >4.7 mg/dL (>1.52 mmol/L). There are three basic causes for elevated serum phosphate concentrations:

- Decreased renal phosphate excretion
- Shift of phosphate from intracellular to extracellular fluid
- Increased intake of vitamin D or phosphate-containing products (orally, rectally, or intravenously)

Elevated phosphate concentrations also may result from reduced PTH secretion, increased body catabolism, and certain malignant conditions (e.g., leukemias and lymphomas).[4,47,48]

Causes. The most common cause of hyperphosphatemia is renal dysfunction, which commonly occurs as the GFR falls below 25 mL/min. CKD results in secondary hyperparathyroidism, which can further reduce renal phosphate elimination. The increase in serum phosphate concentration increases the risk for deposition of insoluble calcium–phosphate complex in soft tissues (i.e., metastatic calcification). This deposition may further reduce the serum concentration of ionized calcium and lead to increased PTH production and release. A sustained period of high PTH levels leads to excessive bone resorption, which will severely weaken its structural integrity.[36,40]

Hyperphosphatemia can be caused by a shift of phosphate from intracellular to extracellular fluid. This shift of phosphate can result from massive cell break down after administering chemotherapy for leukemia or lymphoma, and during rhabdomyolysis and septic shock. In addition, hyperthyroidism can elevate serum phosphate by directly increasing renal tubular phosphate reabsorption.

Clinical diagnosis. Signs and symptoms of hyperphosphatemia commonly result from the accompanying hypocalcemia and hyperparathyroidism (see Hypocalcemia section). Renal function may diminish if hyperphosphatemia is left untreated. In the presence of renal dysfunction, phosphate excretion is further reduced to cause an even greater increase of serum phosphate concentration and a further decline in serum calcium concentration.[36,40,45] (**Minicase 4**.)

Causes of spurious laboratory results. Hemolysis can occur during phlebotomy, which may lead to a falsely elevated serum phosphate concentration. If the serum is not separated soon after phlebotomy, phosphate may be falsely decreased as it is taken up by the cellular components of blood.

Similar to what may occur to specimens for potassium concentration determination, when the blood is allowed to clot

MINICASE 4

Calcium and Phosphate Disorders in a Patient with Chronic Renal Failure

Michael S., a 72-year-old man, had a 1-week history of nausea, vomiting, and general malaise. His appetite has severely decreased over the past two months. He has a longstanding history of uncontrolled hypertension and type 2 diabetes mellitus as well as diabetic nephropathy, retinopathy, and neuropathy.

His physical examination reveals a BP of 160/99 mm Hg, diabetic retinopathic changes with laser scars bilaterally, and diminished sensation bilaterally below the knees. His laboratory values include serum sodium 146 mEq/L, potassium 4.7 mEq/L, chloride 104 mEq/L, total carbon dioxide content 15 mEq/L, SCr 3.2 mg/dL (3.1 mg/dL from one month ago), BUN 92 mg/dL, and random blood glucose of 181 mg/dL. Because of his renal failure, additional laboratory tests were obtained: calcium 7.5 mg/dL, phosphate 9.1 mg/dL, albumin 3.3 g/dL.

Over the next several days, he complains of finger numbness, tingling, and burning of extremities. He also has experienced increasing confusion and fatigue. A neurological examination is positive for both Chvostek and Trousseau signs. Repeated laboratory tests show substantial changes in serum calcium (6.1 mg/dL) and phosphate (10.4 mg/dL). His intact serum PTH is 280 pg/mL (10–65 pg/mL).

QUESTION: Please characterize this patient's calcium and phosphorus disorders?

DISCUSSION: He has three laboratory abnormalities that are related specifically to calcium–phosphate metabolism: (1) hypocalcemia, (2) hyperphosphatemia, and (3) secondary hyperparathyroidism. He is exhibiting classic signs and symptoms of hypocalcemia, such as finger numbness, tingling, burning of extremities, confusion, fatigue, and positive Chvostek and Trousseau signs.

Chronic kidney disease, as seen in this patient, is commonly associated with hypocalcemia, hyperphosphatemia, secondary hyperparathyroidism, and vitamin D deficiency. These calcium–phosphate abnormalities are responsible for the development of renal osteodystrophy. During the early stages of renal failure, renal phosphate excretion begins to decrease. His serum phosphate concentration was increased, and the ionized calcium concentration became reduced, which stimulated the release of PTH and resulted in secondary hyperparathyroidism. The higher concentration of PTH reduced his renal tubular phosphate reabsorption, thereby increasing its excretion. The hyperparathyroidism helped to maintain his serum phosphate and calcium concentrations within normal ranges during the early stage of renal failure (Figure 12-3).

As renal function continues to deteriorate (eGFR <30 mL/min), renal tubules cease to respond adequately to the high serum PTH concentration, resulting in hyperphosphatemia. In response to the hypocalcemia that followed, calcium was mobilized from the bone through the action of PTH. However, such a compensatory response was not sufficient as hypocalcemia and hyperphosphatemia continued. The persistent hyperphosphatemia could inhibit the conversion of calcidiol to calcitriol and further reduce the intestinal calcium absorption capacity. Therefore, hypocalcemia was worsened by the presence of hypovitaminosis D, which subsequently stimulated PTH secretion increasing mobilization of calcium from bone. The metabolic acidosis that is common in renal failure also may have contributed to the negative calcium balance in the bone.

This patient was relatively asymptomatic up to this point, primarily because these laboratory abnormalities developed over a long period of time and allowed the body to compensate. In the presence of nausea and vomiting and the lack of appetite, his oral calcium intake was probably reduced substantially, which might have enhanced his malaise. Because calcium is commonly reported as total calcium and not as the free or ionized fraction, his total serum calcium concentration must be corrected for his low serum albumin value. For every 1 g/dL reduction in serum albumin below 4 g/dL, 0.8 mg/dL should be added to his serum calcium concentration. Therefore, with his serum albumin concentration of 3.3 g/dL, his initial serum calcium value of 7.5 mg/dL is equivalent to a total calcium concentration of about 8.1 mg/dL. He does have true hypocalcemia, although the deficit is mild. It is important to note that his calcium and phosphorus derangements are severe. This patient has demonstrated neurological signs of hypocalcemia. His EKG should be checked to determine if his cardiac rhythm is affected by hypocalcemia.

with the use of nonheparinized tubes, phosphate may leach out of platelets to result in a falsely elevated concentration. In patients with thrombocytosis, phosphate concentrations should, therefore, be obtained from plasma rather than serum samples.

Serum phosphate may vary by 1–2 mg/dL (0.32–0.64 mmol/L) after meals. Meals rich in carbohydrate can reduce serum phosphate; meals with high phosphate contents, such as dairy products, can increase serum phosphate. If accurate assessment of the phosphate concentration is necessary, the blood specimen should be obtained from the patient after fasting.

TRACE ELEMENTS

Copper

Normal range: 70–140 mcg/dL (11–22 µmol/L) (males); 80–155 mcg/dL (13–24 µmol/L) (females) for serum copper, 23–50 mg/dL for ceruloplasmin, 0.47 ± 0.06 mg/g for erythrocyte superoxide dismutase

Physiology

The relationship between copper homeostasis and human diseases was uncovered in 1912 shortly after Wilson disease was described. In the early 1930s, a link between copper deficiency and anemia was suspected, although the hypothesis was not proven at that time. In the 1970s, the physiological functions of copper were better understood and its link to various disease states was better appreciated. An official dietary copper recommendation and adequate daily dietary intake was introduced for the first time in 1979.

Copper plays an integral part in the synthesis and functions of many circulating proteins and enzymes. In addition, copper is an essential factor for the formation of connective tissues, such as the cross-linking of collagen and elastin. Copper also regulates the cellular uptake and physiological functions with iron.[49] In the CNS, copper is required for the formation or maintenance of myelin and other phospholipids. Cuproenzymes (copper-dependent enzymes) are crucial in the metabolism of catecholamines. For example, the functions of dopamine hydroxylase and monoamine oxidase are impaired by copper deficiency. Copper also affects the function of tyrosinase in melanin synthesis, which is responsible for the pigmentation of skin, hair, and eyes. Deficiency of tyrosinase results in albinism. Other physiological functions of copper include thermal regulation, glucose metabolism, blood clotting (e.g., factor V function), and protection of cells against oxidative damage.[50,51]

The normal adult daily intake of copper, based on a typical American diet, is about 2–3 mg. Plant copper is in the inorganic (free ionic) form, while meat (animal) copper is in the form of cuproproteins (copper–protein complex). Inorganic copper is absorbed in the upper portion of the GI tract (stomach and proximal duodenum) under acidic conditions. Cuproprotein copper is absorbed in the jejunum and ileum. Absorption of copper from the GI tract is a saturable process. The oral bioavailability of copper ranges from 15–97% and shows a negative correlation with the amount of copper present in the diet.

Once absorbed, copper is bound to a mucosal copper-binding protein called *metallothionein* (a sulfur-rich, metal-binding protein present in intestinal mucosa). From this protein, copper is slowly released into the circulation, where it is taken up by the liver and other tissues.[50] Animal data suggest that the liver serves as the ultimate depot for copper storage. Copper absorption may be reduced by a high intake of zinc (>50 mg elemental zinc /day), ascorbic acid, and dietary fiber. Zinc may induce the synthesis of intestinal metallothionein and form a barrier to copper ion absorption.[50,51]

The normal adult body contains 75–150 mg of copper, which is significantly lower when compared with other trace elements such as zinc and iron. Approximately one third of the total body copper is found in the liver and brain at high tissue concentrations.[52] Another one third is located in the muscles at low tissue concentrations. The rest is found in the heart, spleen, kidneys, and blood (erythrocytes and neutrophils).[50,52]

In the plasma, copper is highly bound (95%) to ceruloplasmin (also known as ferroxidase I), a blue copper protein.[49] This protein contains six to seven copper atoms per molecule. The fraction of plasma copper associated with ceruloplasmin seems to be relatively constant for the same individual. However, a significant interindividual variation exists. The remainder of the plasma copper is bound to albumin and amino acids or is free.[50,52] Copper is eliminated mainly by biliary excretion (average 25 mcg/kg/day), with only 0.5–3% of the daily intake in the urine.[50]

Ceruloplasmin is considered the most reliable indicator of copper status because of its large and relatively stable binding capacity with plasma copper. Therefore, when evaluating copper status in the body, ceruloplasmin concentration should be assessed together with plasma copper concentration.

Hypocupremia

Although it was thought that copper deficiency is relatively uncommon in humans, more cases have been reported recently in patients after bariatric surgery.[51] *Hypocupremia* usually occurs in infants with chronic diarrhea or malabsorption syndrome, such as after bariatric surgery or intestinal resection, or in low-birth-weight infants fed with milk (rather than formulas).[49,51,53] Premature infants, who typically have low copper stores, are at a higher risk for developing copper deficiency under these circumstances.[50]

Copper deficiency may occur in patients receiving long-term parenteral nutrition. Chronic malabsorption syndromes (e.g., celiac disease and ulcerative colitis), protein-wasting enteropathies, short bowel syndrome, and the presence of significant bowel resection or bypass (e.g., malabsorptive bariatric surgical procedures such as long-limb Roux-en-Y, or jejunoileal bypass) are all potential risk factors resulting in copper deficiency.[49,53] Individuals on a vegetarian diet may be at risk because (1) meat is a major food source of copper, and (2) plant sources often have high-fiber content that may interfere with copper absorption.[49]

Prolonged hypocupremia leads to a syndrome of neutropenia and iron-deficiency anemia, which are correctable with copper.[53] The anemia is normocytic or microcytic and hypochromic. It results mainly from poor iron absorption and ineffective heme incorporation of iron.[45,52] Copper deficiency can affect any systems or organs whose enzymes require copper for proper functioning. As such, copper deficiency may lead to abnormal glucose tolerance, arrhythmias, hypercholesterolemia, atherosclerosis, depressed immune function, defective connective tissue formation, demineralization of bones, and pathological fractures.[51]

Two well-known genetic defects are associated with impaired copper metabolism in humans. Menkes syndrome (also called *kinky-hair syndrome/steely-hair syndrome*) is an X-linked disorder that occurs in 1 out of every 50,000 to 100,000 live births. These patients have defective copper absorption, and are commonly deceased by the age of three. They have reduced copper concentrations in the blood, liver, and brain.[49,51] Most of them are children suffering from slow growth and retardation, defective keratinization and pigmentation of hair, hypothermia, and degenerative changes in the aortic elastin and neurons. Progressive nerve degeneration in the brain results in intellectual deterioration, hypotonia, and seizures. However, anemia and neutropenia, hallmark symptoms of nutritional copper deficiency, are not found in Menkes syndrome. Administration of parenteral copper increases serum copper and ceruloplasmin concentrations but does not have any apparent effect on slowing disease progression.

Wilson disease is an autosomal recessive disease of copper storage. Its frequency is uncertain, but it is believed to be not as common as Menkes syndrome. Wilson disease appears to be associated with altered copper catabolism and excretion of ceruloplasmin copper into the bile. It is associated with elevated urinary copper loss and low plasma ceruloplasmin and low plasma copper concentrations. However, copper deposition occurs in the liver, brain, and cornea. If untreated, significant copper accumulation in these organs will eventually lead to irreversible damage such as cirrhosis and neurological impairment. Interestingly, treatment with dietary adjustment of copper intake does not seem to be effective. Chelation therapy using D-penicillamine is much more effective in preventing copper deposition. Oral zinc supplementation also has been used to reduce copper accumulation.

Hypercupremia

Copper excess, or *hypercupremia*, is not common in humans and usually occurs with a deliberate attempt to ingest large quantities of copper. The exact amount of copper that results in toxicity is unknown. Acute or long-term ingestion of >15 mg of elemental copper may lead to symptomatic copper poisoning.[52] Also, it has been reported that drinking water with 2–3 mg/L of copper is associated with hepatotoxicity in infants. Similar to other metallic poisonings, acute copper poisoning leads to nausea, vomiting, intestinal cramps, and diarrhea.[52] A larger ingestion can result in shock, hepatic necrosis, intravascular hemolysis, renal impairment, coma, and death.[53] Elevated intrahepatic copper concentrations may be present in patients with primary biliary cirrhosis and biliary atresia.[49,50,53] Long-term parenteral nutrition use is also a risk factor for hepatic copper overload. The mechanism is not well-established. Chronic cholestasis secondary to parenteral nutrition-associated liver disease has been suggested as the primary cause. Because copper plays an important role in the neurological system, it has been suggested that copper-induced free radical-induced neurodegeneration may be a contributing factor for Alzheimer disease. At present, there is no known treatment for hypercupremia.

Zinc

Normal range: 50–150 mcg/dL (7.7–23 µmol/L)

Physiology

Next to iron, zinc is the most abundant trace element in the body. It is an essential nutrient that is a constituent of, or a cofactor to, many enzymes. These metalloenzymes participate in the metabolism of carbohydrates, proteins, lipids, and nucleic acids.[50] As such, zinc influences the following[50,53]:

- Tissue growth and repair
- Cell membrane stabilization
- Bone collagenase activity and collagen turnover
- Immune response, especially T-cell mediated response
- Sensory control of food intake
- Spermatogenesis and gonadal maturation
- Normal testicular function

The normal adult body contains 1.5–2.5 g of zinc.[51] Aside from supplementation with zinc capsules, dietary intake is the only source of zinc for humans. Food sources of zinc include meat products, oysters, and legumes.[50] Food-based zinc is largely bound to proteins and released by gastric acid and pancreatic enzymes. Ionic zinc found in zinc supplements is absorbed in the duodenum directly.[50] Foods rich in calcium, dietary fiber, or phytate may interfere with zinc absorption, as can folic acid supplements.[50]

After absorption, zinc is transported from the small intestine to the portal circulation where it binds to proteins such as albumin, transferrin, and other globulins.[50] Circulating zinc is bound mostly to serum proteins; two thirds are loosely bound to albumin and transthyretin, while one third is bound tightly to α-2 macroglobulin.[53] Only 2–3% (3 mg) of zinc is either in free ionic form or bound to amino acids.[50]

Zinc can be found in many organs. Tissues high in zinc include liver, pancreas, spleen, lungs, eyes (retina, iris, cornea, and lens), prostate, skeletal muscle, and bone. Because of their mass, skeletal muscle (60–62%) and bone (20–28%) have the highest zinc contents among the body tissues.[50] Only 2–4% of total body zinc is found in the liver. In blood, 85% is in erythrocytes, although each leukocyte contains 25 times the zinc content of an erythrocyte.[51]

Plasma zinc concentration is a poor indicator of total body zinc store. Because 98% of the total body zinc is present in tissues and end organs, the plasma zinc concentration tends to be maintained by continuous shifting from intracellular sources. Additionally, metabolic stress, such as infection, acute myocardial infarction, and critical illnesses increase intracellular shifting of zinc to the liver and lower serum zinc concentrations, even when total body zinc is normal. Conversely, serum zinc concentrations may be normal during starvation or wasting syndromes due to release of zinc from tissues and cells.[50] Therefore, the serum/plasma zinc concentration alone has little meaning clinically. It has been suggested that the rate of zinc turnover in the plasma provides better assessment of the body zinc status. This may be achieved by measuring 24-hour zinc loss in body fluids (e.g., urine and stool). However, this approach is rarely practical for critically ill patients as renal failure is often present. Alternatively, zinc turnover and mobilization may be determined by adjusting plasma zinc concentrations with serum α-2 macroglobulin and albumin concentrations.[56,57] To more accurately assess the body zinc status, others have suggested monitoring the functional indices of zinc, such as erythrocyte alkaline phosphatase, serum superoxide dismutase, and lymphocyte 5' nucleotidase. However, the clinical validity of these tests remains to be substantiated, especially in patients who are acutely ill.

Zinc undergoes substantial enteropancreatic recirculation and is excreted primarily in pancreatic and intestinal secretions. Zinc is also lost dermally through sweat, hair and nail growth, and skin shedding. Except in certain disease states, only 2% of zinc is lost in the urine.[50]

Hypozincemia

In Western countries, zinc deficiency is rare from inadequate intake. Individuals with serum zinc concentrations below 50 mcg/dL (<7.6 μmol/L) are at an increased risk for developing symptomatic zinc deficiency. It also must be emphasized that serum zinc exhibits a negative acute phase response. The presence of proinflammatory cytokines causes an intracellular and intrahepatic influx of zinc from the serum, which would lead to transient *hypozincemia*. Therefore, serum or plasma zinc concentration alone should not be used to assess zinc status in patients with acute illnesses or any acute inflammatory response. Given the caveats of measuring serum zinc concentrations in certain disease states, response to zinc supplements may be the only way of diagnosing this deficiency. In the presence of chronic diseases, it is difficult to determine if zinc deficiency is clinical or subclinical because of the reduced protein binding.[53] Conditions leading to deficiency may be divided into five classes (**Table 12-12**)[50,53]:

- Low intake
- Decreased absorption
- Increased utilization
- Increased loss
- Unknown causes

The most likely candidates for zinc deficiency are infants; rapidly growing adolescents; menstruating, lactating, or pregnant women; individuals with low meat intake; chronically ill patients who have been institutionalized for extended periods; patients with chronic uncontrolled diarrhea or ostomy output, and those who have been receiving zinc-deficient parenteral nutrition solutions.[53] Acrodermatitis enteropathica is an autosomal, recessive disorder involving zinc malabsorption that occurs in infants of Italian, Armenian, and Iranian heritage. It is characterized by severe dermatitis, chronic diarrhea, emotional disturbances, and growth retardation.[50] Examples of malabsorption syndromes that may lead to zinc deficiency include Crohn disease, celiac disease, and short-bowel syndrome.

Excessive zinc may be lost in the urine (hyperzincuria), as occurs in alcoholism, β-thalassemia, diabetes mellitus, diuretic therapy, nephrotic syndrome, sickle cell anemia, and treatment with parenteral nutrition. Severe or prolonged diarrhea (e.g., inflammatory bowel diseases and graft versus host disease) may lead to significant zinc loss in the stool.[50,53] Patients with end-stage liver disease frequently have depleted zinc storage due to decreased functional hepatic cell mass.

TABLE 12-12. Etiologies of Zinc Deficiency

- Low intake
  - Anorexia
  - Nutritional deficiencies
  - Alcoholism
  - Chronic kidney disease
  - Premature infants
  - Certain vegetarian diets
  - Exclusion of trace elements in parenteral nutrition
- Decreased absorption
  - Acrodermatitis enteropathica
  - Malabsorption syndromes
  - Bariatric surgery
  - Short bowel syndrome
- Increased utilization
  - Adolescence
  - Lactation
  - Pregnancy
- Increased loss
  - Alcoholism
  - β-thalassemia
  - Cirrhosis
  - Diabetes mellitus
  - Diarrhea
  - Diuretic therapy
  - Enterocutaneous fistula drainage
  - Exercise (long term, strenuous)
  - Glucagon
  - Impaired enteropancreatic recycling
  - Nephrotic syndrome
  - Protein-losing enteropathies
  - Sickle cell anemia
- Unknown causes
  - Arthritis and other inflammatory diseases
  - Down syndrome

Because zinc is involved in a diverse group of enzymes, its deficiency manifests in numerous organs and physiological systems (**Table 12-13**).[50] Dysgeusia (lack of taste) and hyposmia (diminished smell acuity) are common. Pica is a pathological craving for specific food or nonfood substances (e.g., geophagia). Chronic zinc deficiency, as occurs in acrodermatitis enteropathica, leads to growth retardation, anemia, hypogonadism, hepatosplenomegaly, and impaired wound healing. Additional signs and symptoms of acrodermatitis enteropathica include diarrhea; vomiting; alopecia; skin lesions in oral, anal, and genital areas; paronychia; nail deformity; emotional lability; photophobia; blepharitis; conjunctivitis; and corneal opacities.[50,53]

Hyperzincemia

Zinc is one of the least toxic trace elements.[53] Clinical manifestations of excess zinc, *hyperzincemia,* occur with chronic, high doses of a zinc supplement. However, patients with Wilson disease who commonly take high doses of zinc rarely show signs of toxicity. This may be explained by the stabilization

TABLE 12-13. Signs and Symptoms of Zinc Deficiency

- Signs
  - Acrodermatitis enteropathica
  - Anemia
  - Anergy to skin test antigens
  - Complicated pregnancy
    - Excessive bleeding
    - Maternal infection
    - Premature or stillborn birth
  - Decreased basal metabolic rate
  - Decreased circulating T_4 concentration
  - Decreased lymphocyte count and function
  - Effect on fetus, infant, or child
    - Congenital defects of skeleton, lungs, and CNS
    - Fetal disturbances
    - Growth retardation
    - Hypogonadism
  - Impaired neutrophil function
  - Impairment and delaying of platelet aggregation
  - Increased susceptibility to dental caries
  - Increased susceptibility to infections
  - Mental disturbance
  - Pica
  - Poor wound healing
  - Short stature in children
  - Skeletal deformities
- Symptoms
  - Acne and recurrent furunculosis
  - Ataxia
  - Decreased appetite
  - Defective night vision
  - Hypogeusia
  - Hyposmia
  - Erectile dysfunction
  - Mouth ulcers

CNS = central nervous system; T_4 = thyroxine.

of serum zinc concentrations during high-dose administration.[50] As much as 12 g of zinc sulfate (>2700 mg of elemental zinc) taken over two days has caused drowsiness, lethargy, and increased serum lipase and amylase concentrations. Nausea, vomiting, and diarrhea also may occur.[50]

Serum zinc concentrations must be measured using nonhemolyzed samples. Erythrocytes and leukocytes, like many other cells, are rich in zinc. When they undergo hemolysis in the tube (e.g., too small a needle is used to draw the sample, tourniquet is too tight, or specimen left standing for too long or is mishandled), these cells release zinc into the specimen in quantities large enough to produce misleading results. This phenomenon also can occur when the specimen is allowed to clot, with the use of nonheparinized tubes.[50]

Manganese

Normal range: Varies depending on assay method, sample (whole blood versus plasma), and age. Whole blood method is generally preferred to detect toxicity.

Physiology

Manganese is an essential trace element that serves as a cofactor for numerous diverse enzymes involved in carbohydrate, protein, and lipid metabolism; protection of cells from free radicals; steroid biosynthesis; and metabolism of biogenic amines.[54] Interestingly, manganese deficiency does not affect the functions of most of these enzymes, presumably because magnesium may substitute for manganese in most instances.[53] In animals, manganese is required for normal bone growth, lipid metabolism, reproduction, and CNS regulation.[51]

Manganese has an important role in the normal function of the brain, primarily through its effect on biogenic amine metabolism. This effect may be responsible for the relationship between brain concentrations of manganese and catecholamines.[54]

The manganese content of the adult body is 10–20 mg. Manganese homeostasis is regulated through control of its absorption and excretion.[54] Plants are the primary source of food manganese because animal tissues have low contents.[54] Manganese is absorbed from the small intestine by a mechanism similar to that of iron.[51] However, only 3% to 4% of the ingested manganese is absorbed. Dietary iron and phytate may affect manganese absorption.[49]

Human and animal tissues have low manganese content.[54] Tissues relatively high in manganese are the bone, liver, pancreas, and pituitary gland.[49,54] Most circulating manganese is loosely bound to the β-1 globulin transmanganin, a transport protein similar to transferrin.[51,53] With overexposure, excess manganese accumulates in the liver and brain, causing severe neuromuscular signs and symptoms.[49]

Manganese is excreted primarily in biliary and pancreatic secretions. In manganese overload, other GI routes of elimination also may be used. Little manganese is lost in urine.[53,54]

Manganese Deficiency

Because of its relative abundance in plant sources, manganese deficiency is rare among the general population.[49] Deficiency normally occurs after several months of deliberate manganese omission from the diet.[53,54] Little is known regarding serum manganese concentrations and the accompanying disease states in humans.[53]

Information from the signs and symptoms of manganese deficiency comes from experimental subjects who intentionally followed low manganese diets for many months. Their signs and symptoms included weight loss, slow hair and nail growth, color change in hair and beard, transient dermatitis, hypocholesterolemia, and hypotriglyceridemia.[54]

Adults and children with convulsive disorders have lower mean serum manganese concentrations than normal subjects, although a cause-and-effect relationship has not been established. However, serum manganese concentrations correlate with seizure frequency.[54] Animals deficient in manganese show defective growth, skeletal malformation, ataxia, reproductive abnormalities, and disturbances in lipid metabolism.[49,53]

Manganese Excess

Manganese is one of the least toxic trace elements.[53] Overexposure primarily occurs from inhalation of manganese compounds (e.g., manganese mines).[54] The excess amount accumulates in the liver and brain resulting in severe neuromuscular manifestations. Symptoms include encephalopathy and profound neurological disturbances mimicking Parkinson disease.[49,53,54] These manifestations are not surprising because metabolism of biogenic amines is altered in both manganese excess and Parkinson disease. Other signs and symptoms include anorexia, apathy, headache, erectile dysfunction, and speech disturbances.[53] Inhalation of manganese products may cause manganese pneumonitis.[54]

Chromium

Average range: serum chromium 0.3–0.9 ng/mL; sample contamination (e.g., use of regular blood collection tubes not designed for trace elements may result in ranges from 2–5 ng/mL)

Physiology

The main physiological role of chromium is as a cofactor for insulin.[55] In its organic form, chromium potentiates the action of endogenous and exogenous insulin, presumably by augmenting its adherence to cell membranes.[49] The organic form is in the dinicotinic acid–glutathione complex or glucose tolerance factor (GTF).[51] Chromium is the metal portion of GTF; with insulin, GTF affects the metabolism of glucose, cholesterol, and triglycerides.[53] Therefore, chromium is important for glucose tolerance, glycogen synthesis, amino acid transport, and protein synthesis. Chromium also is involved in the activation of several enzymes.[51]

The adult body contains an average of 5 mg of chromium.[53] Food sources of chromium include brewer's yeast, spices, vegetable oils, unrefined sugar, liver, kidneys, beer, meat, dairy products, and wheat germ.[50,51] Glucose tolerance factor is present in the diet and can be synthesized from inorganic trivalent chromium (Cr^{+3}) available in food and dietary supplements.[50] Chromium is absorbed via a common pathway with zinc; its degree of absorption is inversely related to dietary intake, varying from 0.5–2%.[49,50] Absorption of Cr^{+3} from GTF is 10–25%, but the absorption is only 1% for inorganic chromium.[51]

Chromium circulates as free Cr^{3+}, bound to transferrin and other proteins, and as the GTF complex.[50,53] Glucose tolerance factor is the biologically active moiety and is more important than total serum chromium concentration.[53] Trivalent chromium accumulates in the hair, kidneys, skeleton, liver, spleen, lungs, testes, and large intestine. Glucose tolerance factor concentrates in insulin-responsive tissues such as the liver.[50,51]

The metabolism of chromium is not well-understood for several reasons[51]:

- Low concentrations in tissues
- Difficulty in analyzing chromium in biological fluids and tissue samples
- Presence of different chromium forms in food

Homeostasis is controlled by release of chromium from GTF and by dietary absorption.[50] The kidneys are the main site of elimination where urinary excretion is constant despite variability in the fraction absorbed.[53] However, excretion increases after glucose or insulin administration.[50, 53] Insulin, or a stimulus for insulin release, can therefore mobilize chromium from its stores. The chromium that is released will then be excreted in the urine. The amount of insulin in the circulation can thus affect the elimination and daily requirement of chromium.[55]

Chromium Deficiency

It is important to stress that the body store of chromium cannot be reliably assessed.[50] Serum or plasma chromium may not be in equilibrium with other pools. As with other trace elements, the risk for developing deficiency may be increased in patients receiving prescribed nourishment low in chromium content (e.g., parenteral nutrition solutions).[55] Marginal deficiencies or defects in utilization of chromium may be present in the elderly, patients with diabetes, or patients with atherosclerotic coronary artery disease.[50] The hepatic store of chromium decreases 10-fold in the elderly, suggesting a predisposition to deficiency. Because chromium is involved in lipid and cholesterol metabolism, its deficiency is a suspected risk factor for the development of atherosclerosis.[50,55]

Hyperglycemia increases urinary losses of chromium. Coupled with marginal intake, a type II diabetic patient is predisposed to chromium deficiency, which can further impair glucose tolerance.[50,55] Finally, multiparous women are at a higher risk than nulliparous women for becoming chromium deficient because, over time. Chromium intake may not be adequate to meet fetal needs and to maintain the mother's body store.[55]

The manifestations of chromium deficiency may involve insulin resistance and impaired glucose metabolism. Such manifestations may present clinically in three stages as the deficiency progresses:

- Glucose intolerance is present but is masked by a compensatory increase in insulin release.
- Impaired glucose tolerance and lipid metabolism are clinically evident.
- Marked insulin resistance and symptoms associated with hyperglycemia are evident.[55]

Chromium supplementation has been shown in diabetic patients to increase insulin sensitivity, improve glucose control, and shorten the QTc interval, suggesting a potential favorable effect on cardiovascular risk. However, there is at present no conclusive support demonstrating the benefit of chromium supplementation in diabetic patients or in those with impaired glucose metabolism.

Chromium deficiency may lead to hypercholesterolemia and become a risk factor for developing atherosclerotic disease.[50] Low chromium tissue concentrations have been associated with increased risk for myocardial infarction and coronary artery disease in both healthy subjects and diabetic patients, although a cause-and-effect relationship has not been established.[55,58]

Chromium Excess

Chromium has very low toxicity. The clinical significance of a high body store of chromium is unknown. Serum chromium concentrations may be increased in asymptomatic patients with metal-on-metal prosthetics.

SUMMARY

Hyponatremia and hypernatremia may be associated with high, normal, or low total body sodium. Hyponatremia may result from abnormal water accumulation in the intravascular space (dilutional hyponatremia), a decline in both extracellular water and sodium, or a reduction in total body sodium with normal water balance. Hypernatremia is most common in patients with either an impaired thirst mechanism (e.g., neurohypophyseal lesion) or an inability to replace water depleted through normal insensible loss or from renal or GI loss. Neurological manifestations are signs and symptoms often associated with sodium and water imbalance. The most common symptom of hyponatremia is confusion. However, if sodium continues to fall, seizures, coma, and death may result. Thirst is a major symptom of hypernatremia; elevated urine specific gravity, indicating concentrated urine, is uniformly observed.

Hypokalemia and hyperkalemia may indicate either a true or an apparent (due to transcellular shifting) potassium imbalance. Hypokalemia can occur due to excessive loss from the kidneys (diuretics) or GI tract (vomiting). The most serious manifestation involves the cardiovascular system (i.e., cardiac arrhythmias). Renal impairment, usually in the presence of high intake, commonly causes hyperkalemia. Like hypokalemia, the most serious clinical manifestations of hyperkalemia involve the cardiovascular system.

Serum chloride concentration may be used as a confirmatory test to identify abnormalities in fluid and acid–base balance. Hypochloremia may be diuretic-induced and results from the concurrent loss of sodium and also contraction alkalosis. Hyperchloremia may develop with the use of parenteral nutrition solutions that have a chloride:sodium ratio >1. Signs and symptoms associated with these conditions are related to the abnormalities in fluid or acid–base balance and underlying causes rather than to chloride itself.

Hypomagnesemia usually results from excessive loss from the GI tract (e.g., nasogastric suction, biliary loss, or fecal fistula) or from the kidneys (e.g., diuresis). Magnesium depletion is usually associated with neuromuscular symptoms such as weakness, muscle fasciculation with tremor, tetany, and increased reflexes. Increased magnesium intake in the presence of renal dysfunction commonly causes hypermagnesemia. Neuromuscular signs and symptoms that are opposite to those caused by hypomagnesemia may be observed.

The most common causes of true hypocalcemia are disorders of vitamin D metabolism and PTH production. Severe hypocalcemia can be a medical emergency and lead to cardiac arrhythmias and tetany, with symptoms primarily involving the neuromuscular system.

The most common causes of hypercalcemia are malignancy and primary hyperparathyroidism. Symptoms often consist of vague GI complaints such as nausea, vomiting, abdominal pain, anorexia, constipation, and diarrhea. Severe hypercalcemia can cause cardiac arrhythmias, which can be a medical emergency.

The most common causes of hypophosphatemia are decreased intake and increased renal loss. Although mild hypophosphatemia is usually asymptomatic, severe depletion (<1 mg/dL or <0.32 mmol/L) is typically associated with muscle weakness, rhabdomyolysis, paresthesia, hemolysis, platelet dysfunction, and cardiac and respiratory failure. The most common cause of hyperphosphatemia is renal dysfunction, often with a GFR below 25 mL/min. Signs and symptoms, if present, primarily result from the ensuing hypocalcemia and hyperparathyroidism.

Hypocupremia is uncommon in adults but can occur in infants, especially those born prematurely. Also susceptible are infants who have chronic diarrhea, malabsorption syndrome, or those whose diet consists mostly of milk. Prolonged hypocupremia results in neutropenia and iron-deficiency anemia that is correctable with copper.

Copper excess is not common and may result from a deliberate attempt to ingest large quantities. Similar to other metallic poisonings, acute copper poisoning leads to nausea and vomiting, intestinal cramps, and diarrhea.

Likely candidates for zinc deficiency are infants; rapidly growing adolescents; menstruating, lactating, or pregnant women; persons with low meat intake; institutionalized patients; and patients receiving parenteral nutrition solutions. Because zinc is involved with a diverse group of enzymes, its deficiency manifests in different organs and physiological systems. Zinc excess develops from chronic, high-dose zinc supplementation. Signs and symptoms include nausea, vomiting, diarrhea, drowsiness, lethargy, and increases in serum lipase and amylase concentrations.

Manganese deficiency can occur after several months of deliberate omission from the diet. Signs and symptoms include weight loss, slow hair and nail growth, color change in hair and beard, transient dermatitis, hypocholesterolemia, and hypotriglyceridemia. Manganese excess primarily occurs through inhalation of manganese compounds (e.g., manganese mines). As a result of manganese accumulation, severe neuromuscular manifestations occur, including encephalopathy and profound neurological disturbances, which mimic Parkinson disease. Inhalation of manganese products may cause manganese pneumonitis.

Chromium deficiency may be found in patients receiving prescribed chronic nutrition regimens that are low in chromium content (e.g., parenteral nutrition solutions). Insulin resistance and impaired glucose metabolism are the main manifestations.

LEARNING POINTS

1. ***What does an abnormal serum electrolyte concentration mean?***

   ANSWER: An isolated abnormal serum electrolyte concentration may not always necessitate immediate treatment because it can be the result of a poor sample (hemolyzed blood sample), wrong timing (immediately after hemodialysis), or other confounding factors. Careful assessment of the patient's existing risk factors, history of illness, and clinical symptoms should be made to correctly interpret a specific laboratory result. Patients with abnormal serum electrolyte concentrations who are also symptomatic, especially with potentially life-threatening clinical presentations such as EKG changes, should be treated promptly. The cause or precipitating factor of the electrolyte abnormality should be identified and corrected, if possible.

2. ***How should we approach a patient who has an abnormal serum sodium concentration?***

   ANSWER: Alteration of serum sodium concentration can be precipitated by sodium alone (either excess or deficiency), or abnormal water regulation. It is important to fully assess the patient's sodium and fluid status, symptoms, physical exam findings, and medical and surgical history for factors that may precipitate sodium disorders. Because the homeostasis of sodium and water is closely regulated by the kidney, it is useful to check urine electrolytes and osmolality to help establish the diagnosis and guide clinical management.

3. ***What are the most common risk factors that can lead to hyperkalemia?***

   ANSWER: The leading cause of hyperkalemia is renal function impairment, especially acute renal insufficiency. Another important cause is drug-induced hyperkalemia (e.g., ACE inhibitors, potassium-sparing diuretic), and high-dietary intake (especially with CKD).

4. ***What is the clinical significance of abnormal serum calcium and phosphorus concentrations?***

   ANSWER: Severe hypocalcemia and hypercalcemia can result in neuromuscular problems. In addition, significant hypercalcemia may cause EKG changes and arrhythmias. Although hyperphosphatemia is not expected to cause any acute problems, severe hypophosphatemia can result in neurologic and CNS manifestations.

   In the presence of chronic hyperphosphatemia, especially in patients with CKD, the risk is increased for phosphorus to bind with calcium to form insoluble complexes which will result in soft tissue and vascular calcification. There is an increasing amount of evidence to show that such vascular calcification can increase the mortality and morbidity of CKD patients. Concurrent hypercalcemia will further increase the serum calcium–phosphorus product and exacerbate the calcification process.

5. ***What is the most common clinical presentation of hypocupremia and what are the causes of copper deficiency?***

   ANSWER: The most common clinical symptoms associated with hypocupremia are neurological symptoms, which may present as ataxia, spasticity, muscle weakness, peripheral neuropathy, loss of vision, anemia, and leukopenia. The most common causes include malabsorption and decreased nutrient consumption.

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QUICKVIEW | Sodium

PARAMETER	DESCRIPTION	COMMENTS
Common reference ranges		
Adults	135–145 mEq/L (135–145 mmol/L)	Useful for assessment of fluid status
Pediatrics: premature neonates	128–148 mEq/L (128–148 mmol/L)	
Pediatrics: older children	138–145 mEq/L (138–145 mmol/L)	
Critical value	>160 or <120 mEq/L (>160 or <120 mmol/L)	Acute changes more dangerous than chronic abnormalities
Natural substance?	Yes	Most abundant cation in extracellular fluid
Inherent activity?	Yes	Maintenance of transmembrane electric potential
Location		
Storage	Mostly in extracellular fluid	
Secretion/excretion	Filtered by kidneys, mostly reabsorbed; some secretion in distal nephron	Closely related to water homeostasis
Major causes of…		
High results	Multiple (discussed in text)	Can occur with low, normal, or high total body sodium
Associated signs and symptoms	Mostly neurological	List 6-2
Low results	Multiple (discussed in text)	Can occur with low, normal, or high total body sodium
Associated signs and symptoms	Mostly neurological	List 6-1
After insult, time to…		
Initial elevation or positive result	Hours to years, depending on chronicity	The faster the change, the more dangerous the consequences
Peak values	Hours to years, depending on chronicity	
Normalization	Days, if renal function is normal	Faster with appropriate treatment
Drugs often monitored with test	Diuretics, ACE inhibitors, aldosterone antagonists, angiotensin II antagonists, ADH analogs	Any drug that affects water homeostasis
Causes of spurious results	None	

ACE = angiotensin-converting enzyme; ADH = antidiuretic hormone.

QUICKVIEW | Potassium

PARAMETER	DESCRIPTION	COMMENTS
Common reference ranges		
Adults and pediatrics	3.8–5 mEq/L (3.8–5 mmol/L)	Age: >10 days old
Critical value	>7 or <2.5 mEq/L (>7 or <2.5 mmol/L)	Acute changes more dangerous than chronic abnormalities
Natural substance?	Yes	Most abundant cation; 98% in intracellular fluid
Inherent activity?	Yes	Control of muscle and nervous tissue excitability, acid–base balance, intracellular fluid balance
Location		
Storage	98% in intracellular fluid	
Secretion/excretion	Mostly secreted by distal nephron	Some via GI tract secretion
Major causes of…		
High results	Renal failure (GFR <10 mL/min)	Especially with increased intake
Associated signs and symptoms	Mostly cardiac	EKG changes, bradycardia, hypotension, cardiac arrest
Low results	Decreased intake or increased loss	Usually combination of the two
Associated signs and symptoms	Affects primarily cardiac system	Table 12-7
After insult, time to…		
Initial elevation or positive result	Hours to years, depending on chronicity	Acute changes can be life-threatening
Peak values	Hours to years, depending on chronicity	
Normalization	Days, if renal function is normal	Faster with appropriate treatment
Drugs often monitored with test	Diuretics, ACE inhibitors, amphotericin B, angiotensin receptor antagonists, cisplatin, trimethoprim	Some drugs are administered as potassium salts Be aware of potassium-sparing medications
Causes of spurious results	Hemolyzed samples (falsely elevated)	High potassium content in erythrocytes

ACE = angiotensin-converting enzyme; EKG = electrocardiogram; GFR = glomerular filtration rate; GI = gastrointestinal.

QUICKVIEW | Chloride

PARAMETER	DESCRIPTION	COMMENTS
Common reference ranges		
Adults and pediatrics	95–103 mEq/L (95–103 mmol/L)	
Critical value		Depends on underlying disorder
Natural substance?	Yes	
Inherent activity?	Yes	Primary anion in extracellular fluid and gastric juice, cardiac function, acid–base balance
Location		
Storage	Extracellular fluid	Most abundant extracellular anion
Secretion/excretion	Passively follows sodium and water	Also influenced by acid–base balance
Major causes of…		
High results	Dehydration Acidemia	
Associated signs and symptoms	Associated with underlying disorder	
Low results	Nasogastric suction Vomiting Serum dilution Alkalemia	
Associated signs and symptoms	Associated with underlying disorder	
After insult, time to…		
Initial elevation or positive result	Hours to years, depending on chronicity	The faster the change, the more dangerous the consequences
Peak values	Hours to years, depending on chronicity	
Normalization	Days, if renal function is normal	Faster with appropriate treatment of underlying disorder
Drugs often monitored with test	Loop diuretics, chloride-containing IV fluids (e.g., saline solution), parenteral nutrition, drugs that cause diarrhea	
Causes of spurious results	Bromides; iodides (falsely elevated)	

IV = intravenous.

QUICKVIEW | Magnesium

PARAMETER	DESCRIPTION	COMMENTS
Common reference ranges		
Adults and pediatrics	1.7 –2.4 mEq/L (0.7 –0.99 mmol/L)	
	1.4–2 mEq/L	
Critical value	>5 or <1 mEq/L (>2.5 or <0.5 mmol/L)	Acute changes more dangerous than chronic abnormalities
Natural substance?	Yes	
Inherent activity?	Yes	Enzyme cofactor, thermoregulation, muscle contraction, nerve conduction, calcium and potassium homeostasis
Location		
Storage	50% bone, 45% intracellular fluid, 5% extracellular fluid	
Secretion/excretion	Filtration by kidneys	3–5% reabsorbed
Major causes of…		
High results	Renal failure	Usually in presence of increased intake
Associated signs and symptoms	Neuromuscular manifestations	Table 12-9
Low results	Excessive loss from GI tract or kidneys	Alcoholism and diuretics
	Decreased intake	
Associated signs and symptoms	Neuromuscular and cardiovascular manifestations including weakness, muscle fasciculation, tremor, tetany, increased reflexes, and EKG abnormalities	More severe with acute changes
After insult, time to…		
Initial elevation or positive result	Hours to years, depending on chronicity	The faster the change, the more dangerous the consequences
Peak values	Hours to years, depending on chronicity	
Normalization	Days, if renal function is normal	Faster with appropriate treatment
Drugs often monitored with test	Diuretics, proton pump inhibitors	
Causes of spurious results	Hemolyzed samples (falsely elevated)	

EKG = electrocardiogram; GI = gastrointestinal.

QUICKVIEW | Calcium

PARAMETER	DESCRIPTION	COMMENTS
Common reference ranges		
Adults	Total calcium: 9.2–11 mg/dL (2.3–2.8 mmol/L)	Approximately half of calcium in the blood is bound to serum proteins; only ionized (free) calcium is physiologically active
	Ionized calcium: 4.6–5.8 mg/dL (1.16–1.45 mmol/L)	
Pediatrics	Total calcium: 8–10.5 mg/dL (2–2.6 mmol/L)	See Chapter 21 for detailed listing of normal ranges based on patient's age
	Ionized calcium: 1.16–1.45 mmol/L	
Critical value	>14 or <7 mg/dL (>3.5 or <1.8 mmol/L)	Also depends on serum albumin and pH values
Natural substance?	Yes	
Inherent activity?	Yes	Preservation of cellular membranes, propagation of neuromuscular activity, regulation of endocrine functions, blood coagulation, bone metabolism, phosphate homeostasis
Location		
Storage	99.5% in bone and teeth	Very closely regulated
Secretion/excretion	Filtration by kidneys	Small amounts excreted into GI tract from saliva, bile, and pancreatic and intestinal secretions
Major causes of…		
High results	Malignancy	Also thiazide diuretics, lithium, vitamin D, teriparatide, and calcium supplements
	Hyperparathyroidism	More severe with acute onset
Associated signs and symptoms	Vague GI complaints neurological and cardiovascular symptoms, and renal dysfunction	
Low results	Vitamin D deficiency Chronic kidney disease Hypoparathyroidism Hyperphosphatemia Pancreatitis Loop diuretics Calcitonin Denosumab	Hypocalcemia due to hypoalbuminemia is asymptomatic (ionized calcium concentration unaffected)
Associated signs and symptoms	Primarily neuromuscular (e.g., fatigue, depression, memory loss, hallucinations, seizures, tetany)	More severe with acute onset
After insult, time to…		
Initial elevation or positive result	Hours to years, depending on chronicity	The faster the change, the more dangerous the consequences
Peak values	Hours to years, depending on chronicity	
Normalization	Days, if renal function is normal	Faster with appropriate treatment
Drugs often monitored with test	Loop diuretics, calcitonin, vitamin D, calcium supplements, phosphate binders	
Causes of spurious results	Hypoalbuminemia	Ionized calcium concentration usually unaffected

GI = gastrointestinal.

QUICKVIEW | Phosphate

PARAMETER	DESCRIPTION	COMMENTS
Common reference ranges		
Adults	2.3–4.7 mg/dL (0.74–1.52 mmol/L)	
Pediatrics	4–7.1 mg/dL (1.3–2.3 mmol/L)	See Chapter 21 for detailed listing of normal ranges based on patient's age
Critical value	>8 or <1 mg/dL (>2.6 or <0.3 mmol/L)	Acute changes more dangerous than chronic abnormalities
Natural substance?	Yes	Most abundant intracellular anion
Inherent activity?	Yes	Bone and tooth integrity, cellular membrane integrity, phospholipid synthesis, acid–base balance, calcium homeostasis, enzyme activation, formation of high-energy bonds
Location		
Storage	Extracellular fluid, cell membrane structure, intracellular fluid, collagen, bone	85% in bone
Secretion/excretion	Filtration by kidneys	Mostly reabsorbed
Major causes of…		
High results	Decreased renal excretion	Renal failure the most common cause
	Extracellular shifting	
	Increased intake of phosphate or vitamin D	
Associated signs and symptoms	Due primarily to hypocalcemia and hyperparathyroidism	See Quickview for calcium (hypocalcemia)
Low results	Increased renal excretion	Also can occur in renal failure
	Intracellular shifting	
	Decreased intake of phosphate or vitamin D	
Associated signs and symptoms	Bone pain, weakness, malaise, hypocalcemia, cardiac failure, respiratory failure	Usually due to diminished intracellular ATP and erythrocyte 2,3-DPG concentrations
After insult, time to…		
Initial elevation or positive result	Usually over months to years	
Peak values	Usually over months to years	
Normalization	Over days with renal transplantation	
Drugs often monitored with test	Vitamin D, phosphate binders	
Causes of spurious results	Hemolyzed samples (falsely elevated) and methotrexate (falsely elevated)	

ATP = adenosine triphosphate.

QUICKVIEW | Copper

PARAMETER	DESCRIPTION	COMMENTS
Common reference ranges		
Adults	70–140 mcg/dL (11–22 μmol/L) (males); 80–155 mcg/dL (13–24 μmol/L) (females)	
Pediatrics	20–70 mcg/dL (3.1–11 μmol/L)	0–6 mo
	90–190 mcg/dL (14.1–29.8 μmol/L)	6 yr
	80–160 mcg/dL (12.6–25.1 μmol/L)	12 yr
Critical value	Not applicable	
Natural substance?	Yes	
Inherent activity?	Yes	Companion to iron enzyme cofactor, hemoglobin synthesis, collagen and elastin synthesis, metabolism of many neurotransmitters, energy generation, regulation of plasma lipid levels, cell protection against oxidative damage
Location		
Storage	One third in liver and brain; one third in muscles; the rest in heart, spleen, kidneys, and blood (erythrocytes and neutrophils)	95% of circulating copper is protein bound as ceruloplasmin
Secretion/excretion	Mainly by biliary excretion; only 0.5–3% of daily intake found in urine	
Major causes of…		
High results	Deliberate ingestion of large amounts (>15 mg of elemental copper) Wilson disease	Uncommon in humans
Associated signs and symptoms	Nausea, vomiting, intestinal cramps, diarrhea	Larger ingestions lead to shock, hepatic necrosis, intravascular hemolysis, renal impairment, coma, and death
Low results	Infants with chronic diarrhea	
	Malabsorption syndromes	
	Decreased intake over months	
	Menkes syndrome	
Associated signs and symptoms	Neutropenia, iron-deficiency anemia, abnormal glucose tolerance, arrhythmias, hypercholesterolemia, atherosclerosis, depressed immune function, defective connective tissue formation, demineralization of bones	Can affect any system or organ whose enzymes require copper for proper functioning
Drugs often monitored with test	Copper supplements, possibly during chronic total parenteral nutrition	Serum copper concentrations not routinely monitored

QUICKVIEW | Zinc

PARAMETER	DESCRIPTION	COMMENTS
Common reference ranges		
Adults and pediatrics	50–150 mcg/dL (7.7–23 μmol/L)	Increased risk for developing symptomatic zinc deficiency
Critical value	<50 mcg/dL (<7.7 μmol/L)	
Natural substance?	Yes	
Inherent activity?	Yes	Enzyme constituent and cofactor; carbohydrate, protein, lipid, and nucleic acid metabolism; tissue growth; tissue repair; cell membrane stabilization; bone collagenase activity and collagen turnover; immune response; food intake control; spermatogenesis and gonadal maturation; normal testicular function
Location		
Storage	Liver, pancreas, spleen, lungs, eyes (retina, iris, cornea, lens), prostate, skeletal muscle, bone, erythrocytes, neutrophils	60–62% in skeletal muscle, 20–28% in bone, 2–4% in liver
Secretion/excretion	Primarily in pancreatic and intestinal secretions; also lost dermally through sweat, hair and nail growth, and skin shedding	Except in certain disease states, only 2% lost in urine
Major causes of…		
High results	Large intake	Uncommon in humans
Associated signs and symptoms	Drowsiness, lethargy, nausea, vomiting, diarrhea, increases in serum lipase and amylase concentrations	
Low results	Low intake (infants) Decreased absorption (acrodermatitis enteropathica) Increased utilization (rapidly growing adolescents and menstruating, lactating, or pregnant women) Increased loss (hyperzincuria)	Rare from inadequate dietary intake
Associated signs and symptoms	Manifests in numerous organs and physiological systems	Table 12-3
Drugs often monitored with test	Zinc supplements, possibly during chronic total parenteral nutrition	Serum zinc concentrations not routinely monitored
Causes of spurious results	Hemolyzed samples; 24-hr intrapatient variability	High zinc content in erythrocytes and neutrophils

QUICKVIEW | Manganese

PARAMETER	DESCRIPTION	COMMENTS
Common reference ranges		
Adults	Varies depending on array method, whether sample is blood or plasma, and patient age	
Pediatrics	2–3 mcg/L (36–55 nmol/L)	
	2.4–9.6 mcg/L (44–175 nmol/L)	Newborn
	0.8–2.1 mcg/L (15–38 nmol/L)	2–18 yr
Critical value	Not applicable	
Natural substance?	Yes	
Inherent activity?	Yes	Enzyme cofactor; carbohydrate, protein, and lipid metabolism; protection of cells from free radicals; steroid biosynthesis; metabolism of biogenic amines; normal brain function Magnesium may substitute for manganese in most instances
Location		
Storage	Bone, liver, pancreas, pituitary gland	Circulating manganese loosely bound to transmanganin
Secretion/excretion	Primarily in biliary and pancreatic secretions; limited excretion in urine	Other GI routes also may be used in manganese overload
Major causes of…		
High results	Primarily through inhalation of manganese compounds, such as in manganese mines	One of least toxic trace elements
Associated signs and symptoms	Encephalopathy and profound neurological disturbances mimicking Parkinson disease	Accumulates in liver and brain
Low results	After several months of deliberate omission from diet	Rare from inadequate dietary intake
Associated signs and symptoms	Weight loss, slow hair and nail growth, hair color change, transient dermatitis, hypocholesterolemia, hypotriglyceridemia	Seen mostly in experimental subjects
Drugs often monitored with test	Manganese supplements, possibly during chronic total parenteral nutrition	Serum manganese concentration not routinely monitored

GI = gastrointestinal.

QUICKVIEW | Chromium

PARAMETER	DESCRIPTION	COMMENTS
Common reference ranges		
Adults	0.3–0.9 ng/mL, serum 0.7–28 ng/mL, whole blood	Analysis of chromium in biological fluids and tissues is difficult
Pediatrics	Unknown	Analysis of chromium in biological fluids and tissues is difficult
Critical value	Unknown	
Natural substance?	Yes	
Inherent activity?	Yes	Cofactor for insulin and metabolism of glucose, cholesterol, and triglycerides
Location		
Storage	Hair, kidneys, skeleton, liver, spleen, lungs, testes, large intestines	Chromium circulates as free Cr3+, bound to transferrin and other proteins, and as an organic complex
Secretion/excretion	Excretion in urine	Circulating insulin may affect excretion
Major causes of…		
Low results	Decreased intake	
Associated signs and symptoms	Glucose intolerance; hyperinsulinemia; hypercholesterolemia; possibly, increased risk of cardiovascular disease	Mainly due to its role as insulin cofactor
Drugs often monitored with test	Chromium supplement, possibly during chronic total parenteral nutrition	Serum chromium concentration not routinely monitored

13

ARTERIAL BLOOD GASES AND ACID–BASE BALANCE

Jeffrey F. Barletta

OBJECTIVES

After completing this chapter, the reader should be able to

- Discuss the chemistry associated with acid–base balance
- Describe the components of an arterial blood gas analysis and their contribution to acid–base physiology
- Compare and contrast the physiologic approach to the Stewart approach for acid–base balance
- Describe the methods used by the human body to maintain acid–base balance
- Evaluate a patient's acid–base status and identify common causes given the clinical presentation, laboratory data, and arterial blood gas data
- List the four simple acid–base disorders, their accompanying laboratory test results, and possible causes
- Describe how the anion gap can be used to determine the primary cause of metabolic acidosis

Acid–base homeostasis is a fundamental component for the maintenance of normal metabolic function. Acid–base disorders, however, are extremely common in the intensive care unit and rapid, careful assessment is required to prevent unwanted morbidity and mortality. This chapter will provide a review of acid–base homeostasis, laboratory tests used to assess acid–base status, and a step-wise approach to classify acid–base disorders and their potential causes.

ACID–BASE CHEMISTRY

An acid is a substance that can donate a proton (e.g., $HCl \rightarrow H^+ + Cl^-$), while a base is a substance that can accept a proton (e.g., $H^+ + NH_3 \rightarrow NH_4^+$). Every acid has a corresponding base and every base has a corresponding acid. Some common acid–base pairs are carbonic acid/bicarbonate, ammonium/ammonia, monobasic/dibasic phosphate, and lactic acid/lactate.

The terms acid*emia* and alkal*emia* are used to describe an abnormal pH. Specifically, acidemia denotes a low pH, while alkalemia denotes a high pH. The terms *acidosis* and *alkalosis,* on the other hand, refer to the process by which either acid or alkali accumulate. It is, therefore, possible to have an acidosis, but not an acidemia. For this to occur (i.e., acidosis without acidemia), a corresponding alkalosis must also be present.

The acidity of a body fluid is determined by the concentration of hydrogen ion (H^+). Normal H^+ concentration is approximately 40 nanoequivalents/L. Because this is expressed in such small amounts (a nanoequivalent is one-millionth of a milliequivalent), acid–base status is measured in pH units using a logarithmic scale. Normal pH is 7.4 with a range of 7.35–7.45. The range of pH values considered compatible with life is 6.8–7.8, which corresponds to a hydrogen ion concentration of only 16 to 160 nEq/L.[1] In general, the body will tolerate acidemia much better than alkalemia. This is due to the fact that as pH decreases, a larger change in H^+ is required for a given change in pH.[2] In alkalemic states, small changes in H^+ can markedly affect pH. Furthermore, with alkalemia, the oxyhemoglobin dissociation curve will shift to the left and hemoglobin is less willing to release oxygen to the tissues (**Figure 13-1**).[3,4]

ARTERIAL BLOOD GASES

Assessment of acid–base status is determined using an arterial blood gas (ABG). Arterial blood reflects how well the blood is being oxygenated by the lungs, while venous blood reflects oxygen consumption by the tissues. It is important that arterial blood is used for these assessments as substantial differences may exist between the two particularly in the setting of critical illness.[5] Note that the "normal ranges" listed below are approximate, and there may be slight variability noted across different references or local laboratory standards.

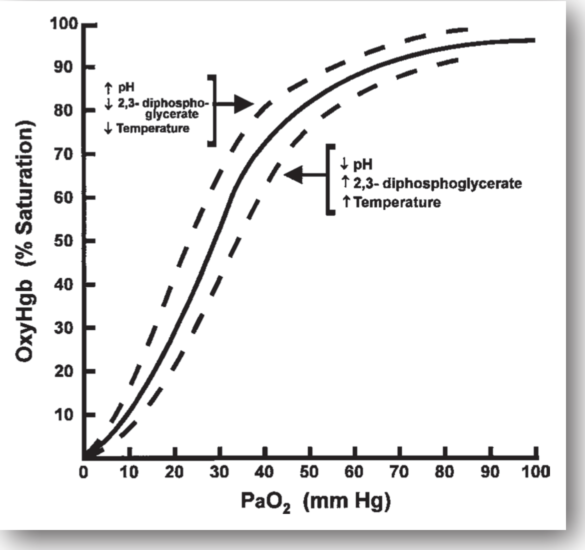

FIGURE 13-1. Oxygen–hemoglobin dissociation curve.

Arterial pH

Normal range: 7.35–7.45

The pH of arterial blood is the first value to consider when using an ABG to assess acid–base status. The pH is inversely related to hydrogen concentration. Generally speaking, pH values below 7.35 represent acidemia, and pH values above 7.45 represent alkalemia with 7.4 being the threshold for categorization during ABG assessment.

It is difficult to identify the pH value that will dictate the urgency whereby treatment must be initiated. Consequences of abnormal pH include arterial vasodilation, venous vasoconstriction, diminished myocardial contractility, impaired hepatic and renal perfusion, decreased oxygen-hemoglobin binding, and coma for acidemia, while cerebral vasoconstriction, reduced contractility, increased oxygen-hemoglobin binding, decreased oxygen delivery, and coma is encountered with alkalemia. These deleterious effects become more prominent when pH is <7.2 or >7.55.[6]

Arterial Partial Pressure of Carbon Dioxide

Normal range: 35–45 mm Hg (4.655–5.985 kPa)

Evaluation of *arterial partial pressure of carbon dioxide* ($PaCO_2$) provides information about the adequacy of lung function in excreting carbon dioxide. The amount of carbon dioxide dissolved in the blood is directly proportional to the concentration of carbonic acid ($PaCO_2 \times 0.03 = H_2CO_3^-$). Elevations in $PaCO_2$, therefore, will contribute to acidosis. Changes to ventilatory status that alter carbon dioxide concentrations will affect carbonic acid concentrations. Specifically, hypoventilation will lead to a higher $PaCO_2$, while hyperventilation will result in a lower $PaCO_2$. Regulation of ventilation is a major mechanism for respiratory compensation in the setting of primary metabolic disorders.

Arterial Partial Pressure of Oxygen

Normal range: 80–100 mm Hg (10.64–13.3 kPa)

Evaluation of the *arterial partial pressure of oxygen* (PaO_2) provides information about the level of oxygenation of arterial blood. The PaO_2 is important because it not only reflects the functional capabilities of the lungs, but also the rate at which oxygen can enter the tissues. Although there is no set cutoff for defining hypoxemia as it is typically relative to metabolic requirements, most would define clinically significant hypoxia at <60 mm Hg. Factors that influence PaO_2 are the amount of ventilation, the fraction of inspired oxygen (FiO_2), the functional capacities of the lung, and the oxyhemoglobin dissociation curve.

The oxyhemoglobin dissociation curve describes the relationship between PaO_2 and oxygen saturation (Figure 13-1). Oxygen saturation is the percentage of hemoglobin binding sites in the bloodstream occupied by oxygen. During states of acidemia, this curve will shift to the right whereby PaO_2 will be higher for a particular level of oxygen saturation. This is reflective of increased unloading of oxygen from hemoglobin. During alkalemia, on the other hand, this curve will shift to the left and higher PaO_2 values are required to maintain oxygen saturation. This will impair oxygen delivery to tissues. Other factors that can influence the oxyhemoglobin dissociation curve are temperature and the amount of 2,3-diphosphoglycerate in the red blood cells.

Although PaO_2 and oxygen saturation are both measurements of oxygenation, it is important to not confuse one with the other. For example, a PaO_2 of 80 mm Hg would typically not be considered abnormal as in most patients this is reflective of an oxygen saturation >93%. An oxygen saturation of 80%, on the other hand, would be considered critical and require immediate intervention. Furthermore, PaO_2 is expressed in mm Hg and values that exceed 100 can exist (especially when supplemental oxygen is administered). Oxygen saturation, however, cannot exceed 100%. Although PaO_2 assessment is crucial for determining pulmonary status, it does not directly impact acid–base balance.

Arterial Bicarbonate

Normal range: 22–26 mEq/L (22–26 mmol/L)

The concentration of *arterial bicarbonate* (HCO_3^-) reported from an ABG is not a direct measurement but calculated using the pH, $PaCO_2$ via the Henderson-Hasselbalch equation. It is important to compare this value (i.e., bicarbonate reading from the ABG) with the total CO_2 content (commonly referred to as *serum bicarbonate*) on an electrolyte panel. Under normal circumstances, the bicarbonate from the ABG is approximately 1.5–3 mEq/L less than the total CO_2 content from a plasma electrolyte panel (higher end of this range for venous samples).[7] Results should be interpreted with caution if this correlation does not exist.

OTHER TESTS ASSOCIATED WITH ACID–BASE INTERPRETATION OR OXYGENATION

Venous Total Carbon Dioxide Content

Normal range: 22–30 mEq/L

The *total carbon dioxide content* refers to the total of all carbon dioxide present in the blood. This consists of bicarbonate, dissolved CO_2, carbonic acid, and carbamino compounds.[7] Because roughly 95% of this is made up of bicarbonate, the term *serum bicarbonate* is often used interchangeably with total carbon dioxide content. Although the name implies that it is a measure of acid (similar to $PaCO_2$), it is important to recognize that this test represents bicarbonate. Increases in total carbon dioxide content will, therefore, contribute to alkalosis.

Anion Gap

Normal range: 3–16 mEq/L (3–16 mmol/L)

The *anion gap* is a calculated value that is an estimate of the relative abundance of unmeasured anions. It is commonly used to determine the possible causes of metabolic acidosis. Anion gap is based on the principles of electrochemical balance; that is, the concentration of negatively charged anions must equal the concentration of positively charged cations. Anion gap is calculated using the following formula (Eq 1):

$$AG = Na^{+} - (Cl^{-} + HCO_3^{-}) \tag{1}$$

Although the normal value for AG can vary, values that exceed 16 mEq/L are generally indicative of anion accumulation (e.g., lactate, pyruvate, acetoacetate). This is often due to lactic acidosis, ketoacidosis, toxic ingestions, or end-stage renal failure.

The AG is largely influenced by plasma albumin; therefore, an adjustment is required when hypoalbuminemia exists. For every decrement of 1 g per deciliter in serum albumin concentration, the calculated AG should be increased by 2.5 (Eq 2)[8]:

$$\text{Corrected AG} = \text{AG} + 2.5\,(4.4\text{ g/dL} - \text{measured albumin}) \tag{2}$$

Anion gap interpretation may also be affected by serum phosphate, magnesium, calcium, and even some β-lactam antibiotics.[9] The influence of these confounding variables, coupled with the heterogeneity of critical illness, has led to questions regarding its diagnostic value.[10-12] Nevertheless, clinicians should consider the AG as one method to assist with potential causes of metabolic acidosis and not the sole factor for decision making at the bedside.

Arterial Oxygen Saturation

Normal range: 93–100%

Arterial oxygen saturation (SaO2) is a measure of the fraction of hemoglobin molecules that are saturated with oxygen. Its relationship between PaO_2 is described using the oxyhemoglobin dissociation curve. Under normal circumstances (e.g., pH, temperature, etc.), a PaO_2 of approximately 60 mm Hg corresponds to an SaO_2 of 90%. Arterial oxygen saturation is a major determinant of arterial oxygen content (CaO_2) (normal range is roughly 17–20 mL/dL; Eq 3) and oxygen delivery (DO_2) (normal range is roughly 950–1150 mL/min or 550–650 mL/min/m^2 when indexed to body surface area; Eq 4):

$$\begin{aligned} CaO_2\,(\text{mL/dL}) = {} & 1.34 \times \text{Hgb (g/dL)} \times SaO_2 \\ & + [PaO_2\,(\text{mm Hg}) \times 0.003] \end{aligned} \tag{3}$$

$$DO_2\,(\text{mL/min}) = CaO_2\,(\text{mL/dL}) \times \text{CO (L/min)} \times 10 \tag{4}$$

(where CO = cardiac output)

Serum Lactate

Normal range: 0.6–2 mmol/L

Serum lactate is a byproduct of anaerobic metabolism; thus, increases reflect inadequate tissue perfusion. The Surviving Sepsis Campaign includes lactate levels >1 mmol/L as part of the diagnostic criteria for sepsis, but levels >4 mmol/L are generally associated with increased mortality.[13,14] Serial lactate levels are often used to assess adequacy of resuscitation, and substantial decreases are associated with improved survival.[15,16]

Venous Oxygen Saturation

Normal range: 65–75%

The *venous oxygen saturation* (SvO_2) in mixed blood describes the balance between systemic oxygen delivery and oxygen uptake. It can also be used as a target for resuscitation. The SvO_2 measurement is taken from a pulmonary artery catheter and is sometimes referred to as *mixed venous oxygenation* because blood from the pulmonary artery is considered to be a mix of venous blood from all tissue beds. Decreases in SvO_2 indicate systemic oxygen delivery is impaired and is often encountered in low flow states (e.g., heart failure, low cardiac index) or anemia.[17] In contrast, SvO_2 may be normal or high in patients with distributive shock.[17,18] This often represents an inability of the tissues to extract the oxygen that has been delivered.

A surrogate of SvO_2 is central venous oxygen saturation ($ScvO_2$). $ScvO_2$ is measured in the superior vena cava and reflects the oxygen saturation of venous blood from only the upper half of the body.[17] It can be obtained from a central venous catheter. In general, $ScvO_2$ is typically 5–7% higher than SvO_2.[19] Target thresholds for $ScvO_2$, therefore, are >70%.[14] High $ScvO_2$ values (i.e., >90%) have been associated with poor outcomes.[20]

ACID–BASE PHYSIOLOGY

The Physiologic Approach: Henderson-Hasselbalch

The physiologic approach is largely composed of the carbonic acid-bicarbonate buffering system and described by the Henderson-Hasselbalch equation (Eq 5):

$$pH = 6.1 + \log [HCO_3^- / (0.03 \times pCO_2)] \qquad (5)$$

In this equation, both HCO_3^- and pCO_2 are independent variables with HCO_3^- representing the base and pCO_2 representing the acid. Disturbances that primarily affect pCO_2 concentrations are called *respiratory,* while those that effect HCO_3^- are called *metabolic.*

The pH is determined not by the absolute values of either but by the ratio of HCO_3^- to pCO_2. That being said, both values may be largely abnormal (indicating an acid–base disorder is present), but pH may be in the normal range. In fact, a common pitfall is assuming an acid–base disorder can exist only when pH is abnormal.

The Stewart Approach

A second theory originated from the principles proposed by Peter Stewart and is based on the concept of strong ion difference (SID) and the laws of electrical neutrality (Eq 6)[21]:

$$SID + H^+ - HCO_3^- - CO_3^{2-} - A^- - OH^- = 0 \qquad (6)$$

$$(\text{where } A^- = \text{dissociated weak acids})$$

This theory states that bicarbonate and hydrogen ions are the dependent variables and represent the effects rather than the causes of acid–base imbalances. The three independent variables that ultimately control blood pH are SID, pCO_2, and the total weak acid concentration.

Strong ions are the ones that are completely dissociated (e.g., Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, lactate) as opposed to weak ions that can exist in both charged and uncharged forms (e.g., albumin, phosphate, HCO_3^-).[22] The SID is the difference between the sum of all the strong cations and strong anions.[11] Because not all strong ions can be measured, the apparent SID (aSID) can be calculated as follows (Eq 7):

$$aSID = (Na^+ + K^+ + Ca^{2+} + Mg^{2+}) - (Cl^- + \text{lactate}) \qquad (7)$$

In a healthy individual, the normal SID is approximately +40 to +42.[22,23] As such, this difference must be counterbalanced with an equal opposing charge obtained from pCO_2 and weak acids (e.g., albumin and phosphate). This is referred to as the *effective SID* (eSID). The difference between the aSID and eSID is called the *strong ion gap* (SIG) and in healthy individuals is equal to zero. When the aSID and eSID are not equal, like in a critically ill patient, the imbalance must be matched by a change in the concentration of another charged entity (HCO_3^-, CO_3^{2-}, OH^-, H^+). This makes hydrogen and bicarbonate the dependent variables in this model.

REGULATION OF ACID–BASE HOMEOSTASIS

The metabolism of carbohydrates and fat results in the production of approximately 15,000 mmol of CO_2 per day. In addition, digestion of proteins and tissue metabolism results in the production of nonvolatile acids. For normal cellular function to occur, hydrogen ion concentration must be maintained within a narrow therapeutic range. In fact, the normal variance of hydrogen ion in extracellular fluid is <10 nEq/L.[2] The three mechanisms the body uses to maintain this tight therapeutic range are buffers, respiratory regulation, and renal regulation.

Buffers

Buffers represent the first line of defense when an acid–base imbalance exists. A buffer is a substance that can absorb or donate hydrogen ions when in the presence of a strong acid or base and minimize resultant changes in pH. The principle buffer system in the body is the bicarbonate/carbonic acid system (Eq 8):

$$HCO_3^- + H^+ \leftrightarrow H_2CO_3 \leftrightarrow CO_2 + H_2O \qquad (8)$$

This system plays a central role because both HCO_3- and CO_2 can be regulated independently. Reactions in this system flow both ways depending on the concentration of each component. In this model, carbonic acid (H_2CO_3) and bicarbonate (HCO_3^-) exist in equilibrium with hydrogen ions. In the presence of carbonic anhydrase, carbonic acid is converted to CO_2. Carbon dioxide concentrations are regulated through ventilation (i.e., the respiratory component), while bicarbonate concentrations are regulated through the kidneys (i.e., the metabolic component).

Other buffer systems that are present are the phosphate buffer system as well as intracellular and extracellular proteins, which function more so as intracellular buffers.

Respiratory Regulation

The second line of defense against acid–base disturbances is the respiratory system. Within minutes of detecting an imbalance, chemoreceptors located in the medulla of the brain can modify ventilation to either retain or eliminate CO_2. Specifically, an increase in ventilation will decrease CO_2, while a decrease in ventilation will increase CO_2. A new steady-state $PaCO_2$ is typically reached within hours.[1]

Renal Regulation

The kidneys maintain acid–base homeostasis by regulating the concentration of bicarbonate in the blood. Approximately 4300 mEq of bicarbonate is filtered to the kidneys each day; all of which must be reabsorbed to maintain normal acid–base balance.[24] Approximately 90% of this reabsorption takes place in the proximal tubule and is catalyzed by carbonic anhydrase (**Figure 13-2**). The remaining 10% is reabsorbed in the more distal segments. Filtered bicarbonate combines with hydrogen

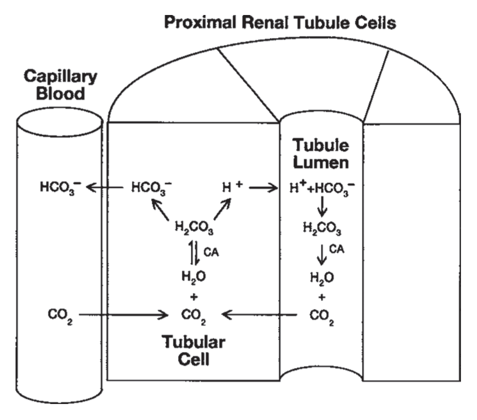

FIGURE 13-2. Renal regulation of acid–base homeostasis.

ions secreted by the renal tubule cell to form carbonic acid. The enzyme carbonic anhydrase, located in the brush border of the renal tubule, catalyzes conversion of carbonic acid to carbon dioxide. The uncharged CO_2 readily crosses the cell membrane and passively diffuses into the renal tubule cell. Inside the cell, CO_2 is converted to carbonic acid in the presence of intracellular carbonic anhydrase. Carbonic acid dissociates into hydrogen ion (which is later secreted into the tubular lumen) and bicarbonate (which is reabsorbed into capillary blood). Drugs that inhibit carbonic anhydrase (e.g., acetazolamide) interfere with this process by blocking the reabsorption of bicarbonate, hence, creating a metabolic acidosis.

The second mechanism used by the kidneys is to excrete the daily load of nonvolatile acids that are produced by the body (approximately 50–100 mEq/day).[24] This is accomplished by hydrogen ions combining with urinary buffers such as phosphates or with ammonia to form ammonium. In general, renal compensation begins approximately 6–12 hours after an acid–base derangement, but full compensation takes roughly three to five days.[25]

ARTERIAL BLOOD GAS INTERPRETATION

Acid–base disorders can be categorized based on the pH derangement (i.e., acidosis and alkalosis) and the primary disorder leading to that derangement (i.e., respiratory and metabolic). There are four classifications for acid–base disorders: metabolic acidosis, metabolic alkalosis, respiratory acidosis, and respiratory alkalosis. The term *respiratory* is used when the primary problem is related to abnormal $PaCO_2$ concentrations, while the term *metabolic* is used when the primary disorder is related to abnormalities in venous bicarbonate. For homeostasis to be maintained, there must be a compensatory response performed by the opposing system (respiratory for primary metabolic disorders and metabolic for primary respiratory disorders). This compensatory response, however, will neither completely correct nor will it overcorrect the primary disorder.

A simple disorder is considered to have a single disturbance with the expected degree of compensation. Mixed disorders, on the other hand, consist of a combination of disturbances that occur simultaneously. Although more than one metabolic disorder can coexist (e.g., metabolic acidosis and metabolic alkalosis), there only can be one respiratory disorder at the same time.

Alternatively, clinicians should think of the following three groups when reviewing causes of acid–base disorders and resultant treatments: (1) iatrogenic (e.g., hyperchloremic metabolic acidosis from saline resuscitation); (2) fixed feature of a pre-existing disease process (e.g., renal failure); or (3) a liable feature of an evolving disease process (e.g., lactic acidosis from shock).[26] Although the conditions listed in each example may all be classified as metabolic acidosis, the treatment plans are inherently different.

STEP-WISE APPROACH FOR ABG ASSESSMENT

Step 1: pH Assessment

The first step in interpreting an ABG is to evaluate the pH and determine if an acidemia or alkalemia exists. A pH lower than 7.4 is acidemia and above 7.4 is alkalemia. As stated earlier, a normal pH does not mean that an acid–base disorder does not exist.

Step 2: Determine Primary Acid–Base Disorder

The second step is to determine if the primary acid–base disorder is respiratory or metabolic. To do this the clinician should perform the following:

1. Pose the question, "If the primary problem were respiratory, would the $PaCO_2$ be high or low?"
2. Use the principles of acid–base physiology to answer the question. Because $PaCO_2$ is considered to be an acid, primary respiratory acidosis occurs when $PaCO_2$ concentrations are high (>40 mm Hg), while primary respiratory alkalosis occurs when $PaCO_2$ concentrations are low.
3. Confirm the actual $PaCO_2$ value from the ABG.

The same approach can then be performed on the metabolic side by evaluating bicarbonate:

1. Pose the question, "If the primary problem were metabolic, would the HCO_3^- be high or low?"
2. Use the principles of acid–base physiology to answer the question. Because HCO_3^- is considered to be a base, primary metabolic acidosis occurs when HCO_3^- concentrations are low (<24 mEq/L), while primary metabolic alkalosis occurs when HCO_3^- concentrations are high.
3. Confirm the actual HCO_3^- value from the ABG.

If the primary problem appears to be both respiratory and metabolic, then a mixed disorder exists.

TABLE 13-1. Summary of Primary Acid–Base Disorders and Their Compensatory Response

DISORDER	pH	PRIMARY ALTERATION	COMPENSATORY ALTERATION	NORMAL COMPENSATORY RESPONSE[a]
Metabolic acidosis	↓	↓ HCO_3^-	↓ $PaCO_2$	Expected $PaCO_2 = (1.5 \times HCO_3^-) + (8 \pm 2)$
Metabolic alkalosis	↑	↑ HCO_3^-	↑ $PaCO_2$	Expected $PaCO_2 = (0.7 \times HCO_3^-) + (21 \pm 2)$
Respiratory acidosis	↓	↑ $PaCO_2$	↑ HCO_3^-	Acute: $\Delta pH = 0.008 \times \Delta PaCO_2$ $\Delta HCO_3^- = \Delta PaCO_2/10$
				Chronic: $\Delta pH = 0.003 \times \Delta PaCO_2$ $\Delta HCO_3^- = 3.5\ (\Delta PaCO_2)/10$
Respiratory alkalosis	↑	↓ $PaCO_2$	↓ HCO_3^-	Acute: $\Delta pH = 0.008 \times \Delta PaCO_2$ $\Delta HCO_3^- = \Delta PaCO_2/5$
				Chronic: $\Delta pH = 0.003 \times \Delta pCO_2$ $\Delta HCO_3^- = \Delta PaCO_2/2$

[a] Conventional units are used in the formulas.

Step 3: Compensatory Response

The third step is to evaluate the degree of compensation for the primary acid–based disturbance. Respiratory compensation can occur quickly after detection of a primary metabolic problem (through adjustment in ventilatory rate), but metabolic compensation for a primary respiratory disorder occurs more slowly. Therefore, when primary respiratory disorders are detected, they are further classified as being either acute or chronic. From there, the expected change in either bicarbonate (for primary respiratory disorders) or $PaCO_2$ (for primary metabolic disorders) can be calculated using the appropriate compensation formula (**Table 13-1**). If the calculated expected value differs substantially from the actual value, then a secondary disorder is present.

Step 4: Conditional Assessments for Metabolic Derangements

If a metabolic acidosis exists, the fourth step is to calculate the anion gap. The anion gap can be used to identify the cause of a metabolic acidosis. Although the threshold for "high" will vary, values above 16 are generally considered "positive." The following disorders have been associated with an anion gap-acidosis: lactic acidosis, ketoacidosis, toxic ingestions, or end-stage renal failure. A classic mnemonic that is used to distinguish causes of anion gap acidosis is MUDPILES: **m**ethanol, **u**remia, **d**iabetic ketoacidosis, **p**araldehyde or propylene glycol, **i**soniazid or iron, **l**actic acid, **e**thylene glycol, and **s**alicylates. A more recent mnemonic is GOLD MARK: **g**lycols (ethylene and propylene), **o**xoproline (associated with chronic acetaminophen ingestion), **l**-lactate (most common measured form of lactic acid), **d**-lactate (typically seen in patients with short gut syndromes), **m**ethanol, **a**spirin, **r**enal failure, and **k**etoacidosis.[27]

If a metabolic alkalosis exists, then the clinician should determine if the disorder is chloride responsive or chloride resistant. This is performed by assessing the urinary Cl^-. Urinary Cl^- concentrations <25 mEq/L are suggestive of chloride-responsive alkalosis, while urinary Cl^- concentrations >40 mEq/L are chloride-resistant.[1] Chloride-responsive alkalosis typically responds to intravenous normal saline (0.9% sodium chloride) administration. Common causes of chloride-responsive alkalosis include vomiting, gastrointestinal losses, gastrointestinal drainage, and diuretics. Chloride-resistant alkalosis, on the other hand, does not respond to normal saline administration and is often reflective of mineralocorticoid excess or severe hypokalemia. Other causes include Bartter syndrome, Cushing syndrome, Gitelman syndrome, severe hypercalcemia, and severe magnesium deficiency.

ACID–BASE DISORDERS

Metabolic Acidosis

Metabolic acidosis is caused by the net retention of nonvolatile acids or loss of bicarbonate. On ABG, both the pH and the serum bicarbonate would be low and the $PaCO_2$ would decrease in an attempt to compensate. This would be manifested by an increase in respirations.

Traditionally, metabolic acidosis is classified by the anion gap that can be used to identify the underlying cause. Metabolic acidosis with an increased anion gap commonly results from increased endogenous organic acid production, while nonanion gap acidosis is often related to extensive loss of bicarbonate. Common causes of anion gap and nonanion gap acidosis are listed in **Table 13-2**. One cause of anion gap acidosis is lactic acidosis, which is further classified as being type A (i.e., hypoxic) or type B (i.e., nonhypoxic). Causes of type A lactic acidosis include septic shock, mesenteric ischemia, hypoxemia, hypovolemic shock, carbon monoxide poisoning, and cyanide toxicity. Type B lactic acidosis can be caused by seizures, intoxication (e.g., salicylate, ethylene glycol, propylene glycol), and medications (Table 13-2). A complete review of the patient's medication list should, therefore, be performed to rule out potential drug-induced causes.

TABLE 13-2. Causes of Metabolic Acidosis

ANION GAP

- Lactic acidosis
  - *Type A:*
    - Septic shock
    - Hypovolemic shock
    - Mesenteric ischemia
    - Hypoxemia
    - Severe anemia
    - Carbon monoxide poisoning
    - Cyanide
  - *Type B:*
    - Medications
      - Nonnucleoside reverse-transcriptase inhibitors
      - Metformin
      - Propofol
      - Linezolid
      - Niacin
      - Isoniazid
      - Iron
      - IV lorazepam (vehicle)
      - Sodium nitroprusside (cyanide)
    - Seizures
    - Diabetes mellitus
    - Malignancy
    - Intoxication
- Poisonings
  - Salicylate
  - Ethylene glycol
  - Propylene glycol
  - Methanol
  - Toluene ingestion
  - Paraldehyde
- Ketoacidosis
  - Diabetic
  - Alcoholic
  - Starvation
  - Metabolic errors
- Renal failure

NONANION GAP

- Gastrointestinal losses
  - Diarrhea
  - Ureteral diversions
  - Fistulas
- Medications and iatrogenic causes
  - Normal saline (excessive doses)
  - Total parenteral nutrition
  - Carbonic anhydrous inhibitors
  - Topiramate
  - Cholestyramine
- Renal tubular acidosis

IV = intravenous.

Nonanion gap acidosis occurs when the decrease in bicarbonate ions corresponds with an increase in chloride ions to maintain electrical neutrality. A common cause of nonanion gap acidosis is hyperchloremic metabolic acidosis due to resuscitation with large volumes of normal saline. Nonanion gap acidosis is also encountered with renal tubular acidosis, excessive gastrointestinal loses (e.g., diarrhea, fistula drainage, ureteral diversion) or iatrogenic causes (e.g., total parenteral nutrition, carbonic anhydrase inhibitors). (**Minicases 1 and 3.)**

Metabolic Alkalosis

Metabolic alkalosis is caused by a net gain of bicarbonate or loss of hydrogen ion from the extracellular fluid. It is characterized on ABG by an increase in both pH and bicarbonate values. Although some respiratory compensation occurs as a result of hypoventilation and CO_2 retention, the degree is relatively minor.

Metabolic alkalosis is delineated into two types: chloride responsive and chloride resistant (**Table 13-3**). A common drug-related cause of metabolic alkalosis is diuretic therapy. Diuretics cause a wasting of Cl^- in association with Na^+ and K^+ without a proportional increase in bicarbonate excretion. In addition, volume depletion leads to hyperaldosteronism, H^+ secretion, and bicarbonate resorption. (**Minicase 4**.)

MINICASE 1

A Patient with Diarrhea

Joe C. is a 25-year-old male who presents to the emergency department with confusion and severe diarrhea. He states that he just returned from vacation where he took a cruise for spring break. His past medical history is noncontributory. Laboratory values and ABG are as follows: pH 7.3, $PaCO_2$ 35 mm Hg, PaO_2 80 mm Hg, HCO_3^- 18 mEq/L, Na 138 mEq/L, K 3.8 mEq/L, Cl 110 mEq/L, total carbon dioxide 20 mEq/L, BUN 40 mg/dL, SCr 1.1 mg/dL, and WBC count 15,000 cells/mm^3.

QUESTION: What acid–base disorder does this patient present with? What is the most likely cause?

DISCUSSION: The pH of 7.3 indicates acidemia. Because both the HCO_3^- and the $PaCO_2$ are low, the primary disorder is metabolic acidosis. This is consistent with the patient's clinical presentation and chief complaint of diarrhea (hence, the loss of bicarbonate). The degree of respiratory compensation can be calculated using the formula $PaCO_2 = 1.5 \times HCO_3^- + 8 \pm 2$. In this example, the actual $PaCO_2$ (35 mm Hg) is within the range for expected $PaCO_2$ (33 – 37 mm Hg), indicating this is a simple disorder. The next step is to calculate the anion gap which is 8 (consistent with a nonanion gap metabolic acidosis). Common causes of nonanion gap metabolic acidosis are hyperchloremic acidosis due to resuscitation with normal saline, renal tubular acidosis, and excessive gastrointestinal loses. Because this patient presents with excessive diarrhea and confusion likely related to the loss of fluids and dehydration, this is the most likely cause. Treatment should consist of fluid resuscitation and evaluation for a possible infectious source for the diarrhea given the recent travel history.

MINICASE 2

A Patient with Acute Respiratory Depression

Alice B. is an 80-year-old female who is admitted to the hospital after suffering a fall and breaking her hip. On hospital day 2, she presents to the operating room to have her hip repaired. Postoperatively, her stay has been relatively unremarkable with the exception of pain control. Upon evaluation, her pain scores are no lower than 8 out of 10 for which she has been receiving IV hydromorphone. On postoperative day 3, a "rapid assessment" code is called because her oxygen saturation falls to 81%, and her RR is 4 breaths/min. The nurse states that Alice B. has been receiving 2 mg of hydromorphone q 2 hr around the clock, and her last dose was approximately 15 minutes ago.

Her remaining vital signs, laboratory results, and ABG are as follows: HR 100 beats/min, BP 110/70 mm Hg, temperature 37.6 °C, Na 136 mEq/L, K 5.1 mEq/L, Cl 98 mEq/L, total carbon dioxide 28 mEq/L, BUN 25 mg/dL, SCr 0.8 mg/dL, ABG pH 7.26, $PaCO_2$ 58 mm Hg, PaO_2 55 mm Hg, and HCO_3^- 26 mEq/L.

QUESTION: What acid–base disorder does this patient present with? What is the most likely cause?

DISCUSSION: The pH of 7.26 indicates acidemia. Because the $PaCO_2$ and HCO_3^- are both high, the primary cause is a respiratory acidosis. This is consistent with her clinical presentation of respiratory distress and inability to eliminate carbon dioxide. With respiratory acidosis, metabolic compensation is delayed and changes in HCO_3^- are often minimal and rarely >31 mEq/L in the acute setting. In this example, the expected degree of compensation is appropriate for an acute disorder. The most likely cause of respiratory acidosis in this case is respiratory depression secondary to narcotic overuse. Treatment should consist of supplemental oxygen and reversal of hydromorphone with naloxone.

TABLE 13-3. Causes of Metabolic Alkalosis

CHLORIDE-RESPONSIVE	CHLORIDE-RESISTANT
Gastrointestinal loses	Mineralocorticoid excess
Vomiting	Exogenous steroids
Gastric drainage	Increased renin/aldosterone states
Nasogastric suctioning	Cushing syndrome
Chloride wasting diarrhea	Liddle syndrome
Diuretics	Bartter syndrome
Posthypercapnia	

Respiratory Acidosis

Respiratory acidosis is usually a direct result of hypoventilation; therefore, any situation associated with decreased respiratory rate can lead to its occurrence and it is important to identify and treat the underlying cause (**Table 13-4**). Laboratory findings consistent with respiratory acidosis are decreased pH and increased $PaCO_2$. Because renal compensation is slower to respond, the increase in bicarbonate only will be modest at first. With respiratory acidosis, the biggest threat to life is not from acidemia but from hypoxia. In patients breathing room air, $PaCO_2$ cannot exceed 80 mm Hg because life-threatening hypoxia may result.[9] (**Minicase 2.**)

MINICASE 3

A Patient with Fever and Hypotension

Jim D. is a 67-year-old male who is admitted to the intensive care unit following a motor vehicle crash. His injuries include fractures to his pelvis, tibia/fibula, and ribs along with a colon perforation, which was surgically repaired on hospital day 1. It is now hospital day 6, and he remains mechanically ventilated due to respiratory failure. Over the next 24 hours, Jim D. develops a temperature of 39.1 °C, and his blood pressure is 88/56 mm Hg. A bolus of 0.9% sodium chloride is administered, and a norepinephrine infusion is initiated.

Pertinent laboratory values are as follows: sodium 142 mEq/L, potassium 4.1 mEq/L, chloride 107 mEq/L, total carbon dioxide 17 mEq/L, BUN 32 mg/dL, SCr 1.3 mg/dL, WBC count 18,000 cells/mm^3, and lactate 6 mEq/L. The arterial blood gas is pH 7.29, $PaCO_2$ 38 mm Hg, PaO_2 70 mm Hg, and HCO_3^- 15 mEq/L.

QUESTION: What acid–base disorder does this patient present with? What is the most likely cause?

DISCUSSION: The pH is 7.29, which indicates acidemia. Because the HCO_3^- is low and the $PaCO_2$ is low, the primary disturbance is metabolic acidosis. The expected degree of respiratory compensation, which is calculated using the formula expected $PaCO_2 = 1.5 \times HCO_3^- + 8 \pm 2$, is less than the actual $PaCO_2$; therefore, a secondary respiratory acidosis exists. Because the primary problem is a metabolic acidosis, the next step is to calculate the anion gap. The anion gap is 18; thus, this is an anion-gap metabolic acidosis. Common causes of anion-gap acidosis are lactic acidosis, ketosis, renal failure, and poisonings. Because this patient has an elevated lactate level and presents with fever and hypotension, the most likely cause is lactic acidosis secondary to septic shock. The treatment plan should consist of fluid resuscitation and vasopressor support (as needed) to restore tissue perfusion along with broad-spectrum antibiotics.

MINICASE 4

A Patient with Large Bowel Obstruction

Cindy G. is a 55-year-old female who is admitted to the hospital with a large bowel obstruction secondary to diverticulitis for which a surgical resection is performed. Postoperative course has been complicated by a prolonged ileus necessitating nasogastric tube insertion and total parenteral nutrition. Additionally, because of extensive fluid accumulation over the course of this admission, furosemide 80 mg twice daily is being administered.

Laboratory results and ABG are as follows: pH 7.51, $PaCO_2$ 45 mm Hg, PaO_2 65 mm Hg, HCO_3^- 33 mEq/L, sodium 145 mEq/L, potassium 4.2 mEq/L, chloride 96 mEq/L, total carbon dioxide 36 mEq/L, BUN 45 mg/dL, SCr 1.2 mg/dL, and urine chloride 7 mEq/L.

QUESTION: What acid–base disorder does this patient present with? What is the most likely cause?

DISCUSSION: The pH is 7.51, which indicates alkalemia. Because the HCO_3^- is high and the $PaCO_2$ is high, the primary problem is metabolic. The degree of compensation by the respiratory side is appropriate as per the formula expected $PaCO_2 = (0.7 \times HCO_3^-) + 21 \pm 2$. Because the primary problem is metabolic alkalosis, the next step is to determine if it is chloride responsive or resistant. This is done by assessing the urinary chloride. Because the urinary chloride is 7 mEq/L, this would be classified as chloride responsive. There are several potential causes of metabolic alkalosis in this patient. Diuretic administration can cause metabolic alkalosis through aldosterone secretion and increased chloride excretion. Loss of gastrointestinal fluid through nasogastric suction leads to a loss of hydrogen and chloride ions. In addition, parenteral nutrition can be an iatrogenic cause of alkalosis if excessive amounts of acetate are provided in the formula.

TABLE 13-4. Causes of Respiratory Acidosis

- Central
  - Opiates
  - Sedatives
  - Stroke
  - Trauma
  - Head injury
  - Status epilepticus
- Perfusion abnormalities
  - Pulmonary embolism
  - Cardiac arrest
- Airway abnormalities
  - Obstruction
  - Asthma
  - Chronic obstructive pulmonary disease (COPD)
  - Pneumonia
  - Pulmonary edema
  - Acute respiratory distress syndrome
- Neuromuscular
  - Brainstem or cervical cord injury
  - Guillain–Barré syndrome
  - Myasthenia gravis
- Total parenteral nutrition

TABLE 13-5. Causes of Respiratory Alkalosis

- CNS-respiratory stimulation
  - Anxiety
  - Pain
  - Fever
  - Sepsis
  - Pregnancy
  - Progesterone derivatives
  - Salicylates
  - Cerebrovascular accidents
- Hypoxemia
  - Pneumonia
  - Congestive heart failure
  - High altitude
  - Pulmonary edema
  - Pulmonary embolism

CNS = central nervous system.

Respiratory Alkalosis

Respiratory alkalosis is characterized by excessive elimination of CO_2 through hyperventilation. Laboratory derangements include increased pH and decreased $PaCO_2$. Renal compensation is characterized by inhibition of bicarbonate reabsorption, which is complete within several days. It is one of the most frequently encountered acid–base disorders and is associated with a number of pathologic conditions (**Table 13-5**).[9]

SUMMARY

Acid–base disorders are highly prevalent in critically patients. Acid–base status is assessed using an ABG and evaluation of pH, $PaCO_2$, and HCO_3^-. Carbon dioxide is the most abundant acid which is controlled through respiratory regulation, while bicarbonate is the most abundant base and concentrations are maintained by the kidney. There are four primary acid–base disturbances which are categorized by the type (i.e., acidosis versus alkalosis) and origin (metabolic versus respiratory). When evaluating for the presence of acid–base disorders, a systematic approach should be utilized using data obtained from the ABG, serum electrolytes, and clinical presentation. Medications are frequently implicated in acid–base disorders; thus, careful assessment of the medication profile is necessary. These skills can be used to assist clinicians with the identification, prevention, and treatment of acid–base disorders.

LEARNING POINTS

1. ***Can an acid–base disorder exist if a patient presents with a normal pH?***

   ANSWER: Yes, an acid–base disorder can still exist even when the pH is within the normal range. pH is determined by the ratio of base to acid as opposed to the individual concentration of one (i.e., acid or base) independently. Therefore, an acidosis can be present without an acidemia if a coexisting alkalosis is present. Conversely, an alkalosis can be present without an alkalemia if a coexisting acidosis is present. In these settings, evaluation of the $PaCO_2$ and HCO_3^- will reveal abnormal values (a pathophysiologic process), but each will offset one another and the net effect on pH will be negligible. Careful evaluation of the patient's history, clinical presentation, physical exam, and laboratory values is necessary to identify the acid–base disorders that are present.

2. ***What are the steps to follow to assess an ABG?***

   ANSWER: The first step is to determine if an acidemia or alkalemia is present. This is done by evaluating the pH. Once the correct categorization is made, the second step is to determine if the primary cause is metabolic or respiratory. This is performed by assessing the $PaCO_2$ and the HCO_3^- on the ABG. Once the primary cause is determined, the opposing side should compensate. The third step is to assess if the degree of compensation is appropriate. If it is not appropriate, then a secondary disorder is present. Finally, if the primary problem is metabolic acidosis or metabolic alkalosis, then the anion gap or urinary chloride, respectively, should be assessed to assist with identification of the possible cause.

3. ***What is the Stewart approach for acid–base homeostasis?***

   ANSWER: The Stewart approach is based on the laws of electroneutrality, whereby the positive charges (e.g., Na^+, K^+, Ca^{2+}, etc.) and negative charges (e.g., Cl^-, HCO_3^-, etc.) must always be equal. In this model, the three factors that influence pH are the SID, the total weak acid concentration, and the $PaCO_2$. Bicarbonate and hydrogen ions represent the effects rather than the cause of acid–base disturbances. This is in contrast to the physiological approach (e.g., Henderson-Hasselbalch equation) whereby pH is determined by bicarbonate and carbon dioxide.

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QUICKVIEW | Venous Total Carbon Dioxide Content (Venous Serum Bicarbonate)

PARAMETER	DESCRIPTION	COMMENTS
Common reference range		
Adults	22–30 mEq/L (22–30 mmol/L)	Venous bicarbonate can be 1–2 mEq/L higher than arterial measure of bicarbonate
Critical value	<10 mEq/L (<10 mmol/L) or >40 mEq/L (>40 mmol/L)	Reference range and critical values may vary based on local laboratory standards
Inherent activity	Yes	Primary substance responsible for buffering acids
Location		
Production	Byproduct of typical cell metabolism	
Storage	Exchanged via circulation	
Secretion/excretion	Renal excretion (reabsorption occurs at proximal tubule)	
Causes of abnormal values		
High	Metabolic alkalosis and respiratory acidosis	Change in HCO_3- is the primary cause of metabolic acid–base disorders; in contrast, change in HCO_3- is the primary method of compensation for respiratory disorders
Low	Metabolic acidosis and respiratory alkalosis	
Signs and symptoms		
High level	Related to primary process	
Low level	Related to primary process	
After event, time to....		
Initial elevation	6–12 hr to initiate compensation	Assumes acute insult
Peak values	None (will rise until pH balanced)	Assumes insult not yet removed
Normalization	3–5 days to complete compensation	Assumes insult removed and nonpermanent damage
Causes of spurious results	Inadvertent venous sampling Delayed time to analysis	

QUICKVIEW | Arterial Partial Pressure of Carbon Dioxide ($PaCO_2$)

PARAMETER	DESCRIPTION	COMMENTS
Common reference range		
Adults	35–45 mg Hg (4.655–5.985 kPa)	
Critical value	>70 mm Hg (>9.31 kPa) (may require mechanical ventilation)	
Inherent activity	Yes	Primary volatile acid in the body
Location		
Production	Generated intracellularly from carbon dioxide and water	
Storage	n/a	
Secretion/excretion	Excreted by the lungs during expiration	
Causes of abnormal values		
High	Respiratory acidosis and metabolic alkalosis	Change is $PaCO_2$ is the primary cause of respiratory acid–base disorders; in contrast, change in $PaCO_2$ is the primary method of compensation for metabolic disorders
Low	Respiratory alkalosis and metabolic acidosis	
Signs and symptoms		
High level	Respiratory failure	
Low level	Related to primary process	
After event, time to….		
Initial elevation	Minutes to hours	Assumes acute insult
Peak values	None	Assumes insult not yet removed
Normalization	Hours to days	Assumes insult removed and nonpermanent damage
Causes of spurious results	Inadvertent venous sampling Delayed time to analysis	Higher carbon dioxide content

Hg = mercury; hr = hours; n/a = not applicable.

14

PULMONARY FUNCTION AND RELATED TESTS

Lori A. Wilken and Min J. Joo

OBJECTIVES

After completing this chapter, the reader should be able to

- Identify common pulmonary function tests and list their purpose and limitations
  a. Spirometry
  b. Peak expiratory flow rate
  c. Body plethysmography
  d. Carbon monoxide diffusion capacity
  e. Specialized tests
    i. Bronchial provocation test
    ii. Six-minute walk test
    iii. Pediatric pulmonary function testing
    iv. Carbon monoxide breath test
    v. Exhaled nitric oxide breath test
- Describe how pulmonary function tests are performed and discuss factors affecting the validity of the results
- Interpret commonly used pulmonary function tests, given clinical information
- Discuss how pulmonary function tests provide objective measurement to aid in the diagnosis of pulmonary diseases
- Discuss how pulmonary function tests assist with monitoring efficacy and toxicity of various drug therapies

Pulmonary function tests (PFTs) provide objective and quantifiable measures of lung function and are useful in the diagnosis, evaluation, and monitoring of respiratory disease. Diagnosis and monitoring of many pulmonary diseases, including diseases of gas exchange, often require measurement of the flow or volume of air inhaled and exhaled by the patient. Spirometry, a test that measures the movement of air into and out of the lungs during various breathing maneuvers, is the most frequently used PFT. Clinicians use spirometry to aid in the diagnosis of respiratory diseases such as asthma and chronic obstructive pulmonary disease (COPD). Other tests of lung function include lung volume assessment, carbon monoxide diffusion capacity (DLCO), exercise testing, and bronchial provocation tests. PFTs can be used to monitor lung function after thoracic radiation, lung transplantation, or during administration of medications with potential toxicity to the lungs. Arterial blood gases (ABGs) can be measured with PFTs and are useful to assess lung function (interpretation of ABGs is discussed in Chapter 13). This chapter discusses the mechanics and interpretation of PFTs.

ANATOMY AND PHYSIOLOGY OF LUNGS

The purpose of the lungs is to take oxygen from the atmosphere and exchange it for carbon dioxide in the blood. The movement of air in and out of the lungs is called *ventilation;* the movement of blood through the lungs is termed *perfusion*.

Air enters the body through the mouth and nose and travels through the pharynx to the trachea. The trachea splits into the left and right main stem bronchi, and these bronchi deliver inspired air to the respective lungs. The left and right lungs are in the pleural cavity of the thorax. These two spongy, conical structures are the primary organs of respiration. The right lung has three lobes, while the left lung has only two lobes, thus leaving space for the heart. Within the lungs, the main bronchi continue to split successively into smaller bronchi, bronchioles, terminal bronchioles, and finally alveoli. In the alveoli, carbon dioxide is exchanged for oxygen across a thin membrane separating capillary blood from inspired air.

The thoracic cavity is separated from the abdominal cavity by the diaphragm. The diaphragm, a thin sheet of dome-shaped muscle, contracts and relaxes during breathing. The lungs are contained within the rib cage but rest on the diaphragm. Between the ribs are two sets of intercostal muscles, which attach to each upper and lower rib. During inhalation, the intercostal muscles and the diaphragm contract, which enlarges the thoracic cavity. This action generates a negative intrathoracic pressure, allowing air to rush in through the nose and mouth down into the pharynx, trachea, and lungs. During exhalation, these muscles relax, and a positive intrathoracic pressure causes air to be pushed out of the lungs. Normal expiration is a passive process that results from the natural recoil of the expanded lungs. However, in people with rapid or labored breathing or obstruction, the accessory muscles and abdominal muscles often must contract to help force air out of the lungs more quickly or completely.

The ability of the lungs to expand and contract to inhale and exhale air is affected by the compliance of the lungs, which is a measure of the ease of expansion of the lungs and thorax. Processes that result in scarring of lung tissue (e.g., pulmonary fibrosis) can decrease compliance, thus decreasing the flow and volume of air moved by the lungs and increase the work to breathe. The degree of ease in which air travels through the airways is known as *resistance*. The length and radius of the airways as well as the viscosity of the gas inhaled determine resistance. A patient with a high degree of airway resistance may not be able to take a full breath in or to exhale fully (some air may become trapped in the lungs).

To have an adequate exchange of the gases, there must be a matching of ventilation (V) and perfusion (Q) at the alveolar level. An average V:Q ratio, determined by dividing total alveolar ventilation (4 L/min) by cardiac output (5 L/min), is 0.8. A mismatch of ventilation and perfusion may result from a shunt or dead space. A shunt occurs when there is flow of blood adjacent to alveoli that are not ventilated. This could be physiologic (e.g., at rest some alveoli are collapsed or partially opened but perfused) or pathologic when alveoli are filled with fluid (e.g., heart failure), cellular debris (e.g., pneumonia) or collapsed (e.g., atelectasis). A shunt can also occur when airways are obstructed by mucus or collapse on exhalation (e.g., COPD). In a shunt, blood moves from the venous circulation to the arterial circulation without being oxygenated.

Dead space occurs when there is ventilation of functional lung tissue without adjacent blood flow for gas exchange. Dead space can be physiologic (e.g., the trachea) or pathologic due to obstruction of blood flow (e.g., pulmonary embolism). There are a couple of mechanisms that the body uses to normalize the V:Q ratio such as hypoxic vasoconstriction and bronchoconstriction. When the V:Q ratio is low, hypoxic vasoconstriction leads to decreased perfusion to the hypoxic regions of the lungs, thus redirecting perfusion to functional areas of the lungs, which leads to an increase in the V:Q ratio. When the V:Q ratio is high, the bronchi constrict in areas that are not well perfused. This leads to a decrease in the amount of ventilation to areas that are not well perfused, a decrease in the amount of alveolar dead space, and a decrease in the V:Q ratio.

For the respiration process to be complete, gas diffusion must occur between the alveoli and the pulmonary capillaries. By the diffusion mechanism, gases equilibrate from areas of high concentration to areas of low concentration. Hemoglobin (Hgb) releases carbon dioxide and adsorbs oxygen as it diffuses through the alveolar walls. If these walls thicken, diffusion is hampered potentially causing carbon dioxide retention, hypoxia, or both. Membrane formation with secondary thickening of the alveolar wall may result from an acute or chronic inflammatory process such as interstitial pneumonia and pulmonary fibrosis. The pulmonary diffusing capacity is also reduced in the presence of a V:Q mismatch, loss of lung surface areas (e.g. emphysema, lung resection), or decrease in oxygen carrying capacity (e.g., anemia). The various PFTs can measure airflow in or out of the lungs, indicate how much air is in the lungs, and provide information on gas diffusion, or specific changes in airway tone or reactivity.

CLINICAL USE OF PULMONARY FUNCTION TESTING

PFTs are useful in many clinical situations. They aid in the diagnostic differentiation of various pulmonary diseases. For example, with obstructive lung diseases (e.g., asthma or COPD), the underlying pathophysiology is a reversible or persistent blockage in the airways. Obstructive diseases usually decrease the flow rate of air (liters/min) but have a lesser impact or no impact on the total volume per breath. In restrictive diseases (e.g., kyphosis or sarcoidosis), the lungs are limited in the amount of air they can contain. Restrictive diseases usually decrease the total volume of air per breath with little impact or no impact on the flow rate of air. **Table 14-1** summarizes common pulmonary disease states with PFT results.

In addition, serial PFTs allow tracking of the progression of pulmonary diseases and the need for or response to various treatments. They also help to establish a baseline of respiratory function prior to surgical, medical, or radiation therapy. Subsequent serial measurements then aid in the detection and tracking of changes in lung function caused by these therapies. Similarly, serial PFTs can be used to evaluate the risk of lung damage from exposure to environmental or occupational hazards. **Table 14-2** summarizes the selected uses of PFTs.

PULMONARY FUNCTION TESTS AND MEASUREMENTS

There are no normal ranges for PFTs that can be applied to everyone. In general, PFTs use equations based on the individual's age, height, and sex to calculate predicted normal values from the population. The individual's measurement is then compared to the predicted population measurement. If the individual's measurement falls below the predicted measurement, this information is used in the diagnostic assessment of the patient.

Spirometry

Spirometry is a PFT that helps detect airway obstruction, manifested in asthma or COPD. Spirometry measures the maximum amount of air that can be exhaled by the patient after complete inhalation. The physical forces of the airflow and the total amount of air inhaled and exhaled are converted by transducers to electrical signals, which are displayed on a computer screen.

During this maneuver, a *volume-time curve*—a plot of the volume exhaled against time—and a *flow-volume curve* or *flow-volume loop*—a diagram with flow (L/sec) on the vertical axis and volume expired on the horizontal axis (L)—are generated as the report (**Figure 14-1**). After the data are generated, the patient's spirometry results are generally compared to the predicted values for people of similar age, height, and sex. The predicted values are data gathered from the National Health and Nutrition Examination Survey, the Centers for Disease Control and Prevention, and the National Center for Health Statistics. The flow-volume curve is visually

TABLE 14-1. Pulmonary Disease States and Common PFT Results

			COMMON PFT RESULTS				
PULMONARY ABNORMALITY	**PATHOPHYSIOLOGY**	**DISEASE STATE EXAMPLES**	**FEV_1/FVC**	**FEV_1**	**FVC**	**RV**	**TLC**
Obstructive lung disease, chronic	Irreversible (e.g., airway collapse, mucus)	ACOS, COPD, cystic fibrosis, bronchiectasis	Decreased	Decreased	Normal or decreased	Normal or increased	Normal or increased
Obstructive lung disease, reversible and stable	Reversible (e.g., bronchoconstriction)	Asthma	Normal	Normal	Normal	Normal	Normal
Restrictive lung disease	Parenchymal infiltration or fibrosis	Idiopathic pulmonary fibrosis, interstitial pneumonias, sarcoidosis	Normal or increased	Decreased	Decreased	Decreased	Decreased
	Loss of lung volume	Pneumothorax, pneumonectomy, pleural effusions					
	Extrathoracic compression	Kyphosis, morbid obesity, ascites, chest wall deformities, pregnancy					
	Neuromuscular causes	Guillain–Barré syndrome, myasthenia gravis, muscular dystrophy, amyotrophic lateral sclerosis					
Mixed obstructive and restrictive	Combinations of restrictive and obstructive processes	Both restrictive and obstructive diseases	Decreased or normal	Decreased	Decreased	Increased, normal, or decreased	Decreased

ACOS = asthma–chronic obstructive pulmonary disease overlap syndrome; COPD = chronic obstructive pulmonary disease; FEV_1 = forced expiratory volume in one second; FVC = forced vital capacity; PFT = pulmonary function test; RV = residual volume.

TABLE 14-2. Selected Uses of PFTs

Diagnosis
Evaluate signs and symptoms of respiratory disease
Screen at risk individuals for pulmonary disease
Evaluation
Assess the health status before initiating physical activity or rehabilitation
Determine preoperative risk of having pulmonary related issues during surgery
Monitoring
Describe the course of lung function from a respiratory disease
Monitor respiratory changes for occupational or environmental exposure to toxins
Assess therapeutic drug effectiveness (e.g., inhaled corticosteroids or bronchodilators for asthma)
Monitor adverse drug effects on pulmonary function (e.g., amiodarone)

PFTs = pulmonary function tests.

useful for diagnosing airway obstruction. Spirometry is recommended in patients over the age of 40 years with any of the following characteristics: presence of risk factors including cigarette smoking or exposure to occupational dust or chemicals, symptoms of breathlessness, chronic sputum production, or chronic cough.[2] Once diagnosed with COPD, spirometry can be used on an annual basis to monitor disease state severity. Use of spirometry for asthma is recommended at the time of diagnosis, after the initiation of medications and stabilization of symptoms, during periods of uncontrolled asthma, and at least every one to two years to monitor asthma control.[3]

Spirometry Measurements

Spirometry routinely assesses forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), and FEV_1/FVC.

Forced Vital Capacity

The FVC is the total volume of air, measured in liters, forcefully and rapidly exhaled in one breath (from maximum inhalation to maximum exhalation). When the full inhalation-exhalation procedure is repeated slowly—instead of forcefully and rapidly—it is called the *slow vital capacity* (SVC). This value is the maximum amount of air exhaled after a full and complete inhalation. In patients with normal airway function, FVC and SVC are usually similar and constitute the *vital capacity* (VC). In patients with diseases such as COPD, the FVC may be lower than the SVC due to collapse of narrowed or floppy airways during forced expiration. Because of this, some interpretive strategies recommend using the FEV_1/SVC ratio to determine the presence of airway obstruction especially for pronounced airflow limitation.[2]

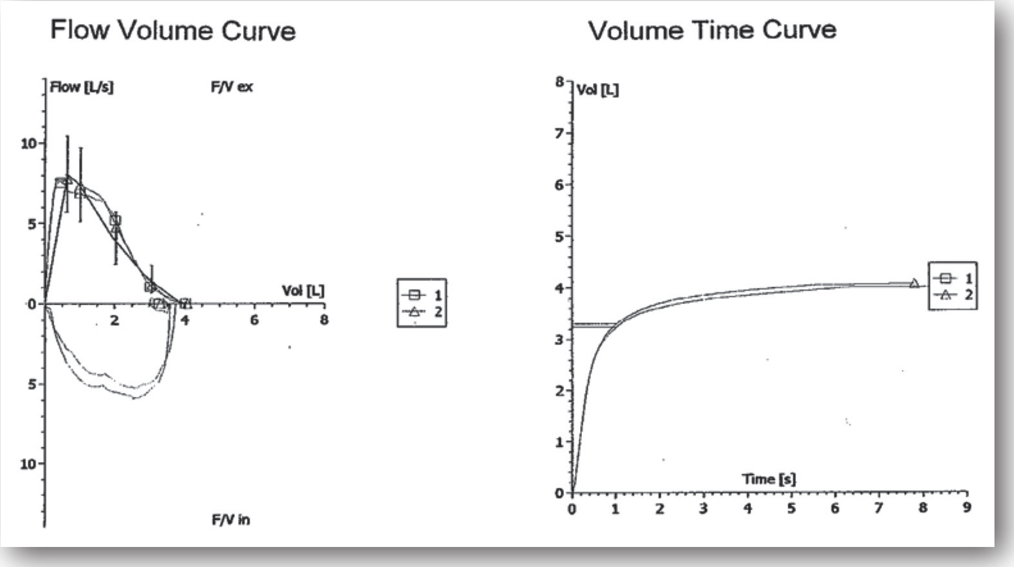

FIGURE 14-1. **The flow-volume curve and volume-time curve from an effort meeting ATS acceptability criteria. The flow-volume curve has a deep inspiratory effort with a sharp complete expiratory flow. The volume-time curve demonstrates both a plateau and an exhalation time of greater than six seconds.**

Forced Expiratory Volume in One Second

The full, forced inhalation-exhalation procedure was already described as the FVC. During this maneuver, the computer can discern the amount of air exhaled at specific time intervals of the FVC. By convention, $FEV_{0.5}$, FEV_1, FEV_3, and FEV_6 are the amounts of air exhaled after one-half, one, three, and six seconds, respectively. Usually, a patient's value is described in liters and as a percentage of a predicted value based on population normal values adjusted for age, height, and sex. Of these measurements, FEV_1 has the most clinical relevance, primarily as an indicator of airway function. A value ≥80% of the predicted normal value is considered normal. Normal values are often seen in patients with reversible airway obstruction when the disease is mild or well controlled.

FEV_1/FVC

The ratio of FEV_1 to the FVC is used to estimate the presence and amount of obstruction in the airways. This ratio indicates the amount of air mobilized in one second as a percentage of the total amount of movable air. Normal, healthy individuals can exhale approximately 50% of their FVC in the first one-half second, about 80% in one second, and about 98% in three seconds. Patients with obstructive disease usually show a decreased ratio, and the actual percentage reduction varies with the severity of obstruction. In COPD, the Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines define chronic airway obstruction as an FEV_1/FVC ratio <70% for both men and women.[2] **Table 14-3** summarizes the definitions of airway obstruction severity for asthma and COPD based on age. **Minicase 1** discusses how spirometry is used to diagnose COPD.

Spirometry alone is not sufficient to diagnose or assess asthma severity. Frequency of asthma symptoms and quick-relief medication use is also necessary to assess asthma severity. Generally, the FEV_1/FVC is normal (or high) in patients with restrictive diseases. In mild restriction, the FVC alone may be decreased resulting in a high ratio. Often in restrictive lung disease, both the FVC and FEV_1 are similarly reduced compared to predicted values resulting in a normal ratio.

Forced Expiratory Flow

Forced expiratory flow (FEF) measures airflow rate during forced expiration. Although FEV measures the volume of air per specific unit of time at the beginning of expiration, FEF measures the rate of air movement during a later portion. The FEF from 25% to 75% of VC is known as $FEF_{25–75}$. This test is thought to measure the flow rate of air in the medium and small airways (bronchioles and terminal bronchioles), but it is not used clinically. The flow from 75% to 100% of VC (i.e., the end of expiration) is called *alveolar airflow*. This parameter may markedly diminish as airways collapse with increased intrathoracic pressure. Such pressure occurs in severe acute asthma when large obstructions are present in terminal bronchioles.

Flow-Volume Curves

Figure 14-2 shows several *flow-volume curves* where the expiratory flow is plotted against the exhaled volume. As explained earlier, these curves are graphic representations of inspiration and expiration. The shape of the curve indicates both the type of disease and the severity of obstruction. Obstructive changes result in decreased airflow at lower lung volumes, revealing a characteristic concave appearance. In obstructive cases, the loop size is similar to that of a healthy individual unless there is severe, acute obstruction. Restrictive changes result in a shape similar to that of a healthy individual, but the size is considerably smaller. The flow-volume loop also reveals

TABLE 14-3. Severity of Airway Obstruction for Asthma and COPD Based on Spirometry Results[a,b]

	INTERMITTENT	MILD	MODERATE	SEVERE	VERY SEVERE
Asthma 5–11 years old	Normal FEV_1 between exacerbations FEV_1 >80% predicted FEV_1/FVC >85%	FEV_1 ≥80% predicted FEV_1/FVC >80%	60 ≥FEV_1<80% predicted FEV_1/FVC 75–80%	FEV_1 <60% predicted FEV_1/FVC <75%	n/a
Asthma 12–19 years old	Normal FEV_1 between exacerbations FEV_1 >80% predicted FEV_1/FVC ≥85%	FEV_1 ≥80% predicted FEV_1/FVC ≥85%	60 ≥FEV_1<80% predicted FEV_1/FVC 80%	FEV_1 <60% predicted FEV_1/FVC <80%	n/a
Asthma 20–39 years old	Normal FEV_1 between exacerbations FEV_1 ≥80% predicted FEV_1/FVC ≥80%	FEV_1 ≥80% predicted FEV_1/FVC ≥80%	60 ≥FEV_1<80% predicted FEV_1/FVC 75%	FEV_1 <60% predicted FEV_1/FVC <75%	n/a
Asthma 40–59 years old	Normal FEV_1 between exacerbations FEV_1 ≥80% predicted FEV_1/FVC ≥75%	FEV_1 ≥80% predicted FEV_1/FVC ≥75%	60 ≥FEV_1<80% predicted FEV_1/FVC 70%	FEV_1 <60% predicted FEV_1/FVC <70%	n/a
Asthma 60–80 years old	Normal FEV_1 between exacerbations FEV_1 ≥80% predicted FEV_1/FVC ≥70%	FEV_1 ≥ 80% predicted FEV_1/FVC ≥70%	60 ≥FEV_1<80% predicted FEV_1/FVC 65%	FEV_1 <60% predicted FEV_1/FVC <65%	n/a
COPD	n/a	FEV_1 ≥80% predicted FEV_1/FVC <70% of predicted	50≤ FEV_1 <80% predicted FEV_1/FVC <70% of predicted	30 ≤FEV_1 <50% predicted FEV_1/FVC <70% of predicted	FEV_1 <30% predicted FEV_1/FVC <70% of predicted

COPD = chronic obstructive pulmonary disease; FEV_1 = forced expiratory volume in one second; FVC = forced vital capacity; n/a = not applicable.

[a] See references 2, 3, and 4 for more information.

[b] FEV_1 in this table is the postbronchodilator FEV_1 percent of predicted normal.

MINICASE 1

USING SPIROMETRY TO DIAGNOSE COPD

Patricia C. is a 64-year-old female with COPD. She was diagnosed 10 years ago and quit smoking cigarettes 5 years ago. She previously smoked one to three packs of cigarettes each day for the past 45 years. Her previous work experience did not involve chemical or dust exposures. She has a daily cough with clear phlegm and becomes short of breath when she tries to climb up a flight of stairs, which requires her to use her albuterol inhaler. She does not have any allergies or identified allergic triggers.

QUESTION: How do the results from this patient's spirometry test support the diagnosis of COPD?

DISCUSSION: A postbronchodilator measurement for FEV_1/FVC <70% is consistent with COPD using the GOLD criteria in the right clinical setting. She has a postbronchodilator FEV_1/FVC of 56.8% consistent with a diagnosis of COPD. In addition, her clinical picture substantiates this diagnosis: significant smoking history (risk factor), shortness of breath on exertion, productive cough, and no allergic triggers. A postbronchodilator FEV_1 of 54.2% of her predicted is considered moderate airflow obstruction. The difference between her reading and a typical patient with asthma is persistent airflow obstruction as evidenced by the postbronchodilator FEV_1/FVC ratio. Patients only with asthma may normalize their postbronchodilator FEV_1/FVC ratio. Although persistently low postbronchodilator FEV_1/FVC can be seen in chronic obstructive asthma, the manifestation of shortness of breath with exertion only, limited triggers, and no history of asthma makes this less likely.

		PREBRONCHODILATOR		POSTBRONCHODILATOR		
PFT	PREDICTED	MEASURED	% PREDICTED	MEASURED	% PREDICTED	% CHANGE
FVC (L)	2.74	2.04	74.33	2.06	75.2	0.98
FEV_1 (L)	2.16	1.19	55.11	1.17	54.2	−2.00
FEV_1/FVC (%)		58.5		56.8		−2.95

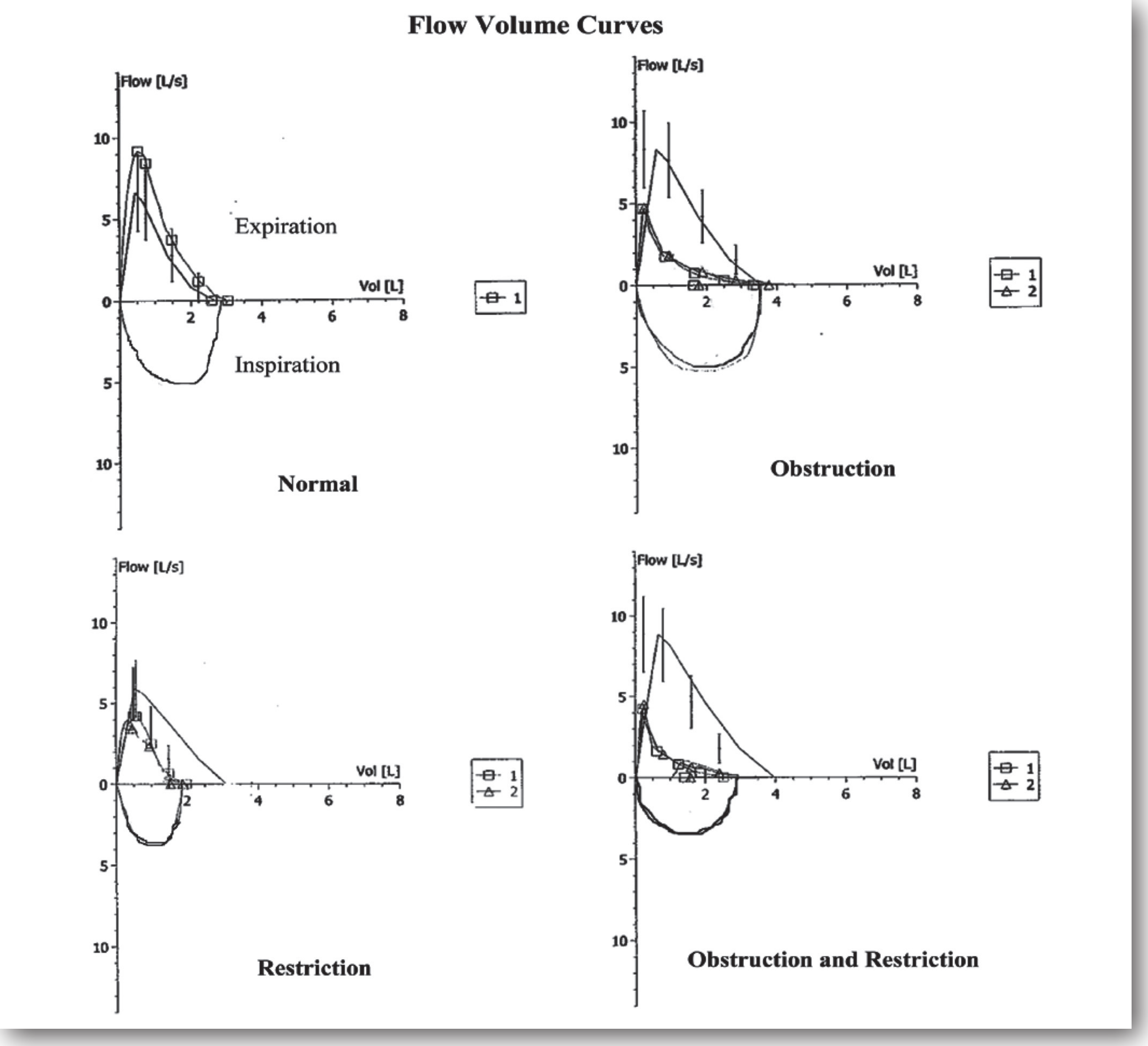

FIGURE 14-2. Flow-volume curves seen with obstructive and restrictive pulmonary diseases. This figure illustrates the flow-volume curves observed for normal adults, obstructive, restrictive, and mixed disease. Flow-volume curve number 1 represents baseline spirometry. Flow-volume curve number 2, if present, is a repeat of the spirometry after a bronchodilator is administered. (Refer to bronchodilator studies under Airway Reactivity Tests.) The curve with the vertical lines represents the predicted normal values. In these examples, there is no improvement in the flow-volume curves after bronchodilator administration. The normal flow-volume curve shows that the curve is larger than expected based on predicted values and are, therefore, normal. Concavity of the expiratory portion of the curve, consistent with limitation in flow compared to the predicted, is illustrated in the obstruction flow-volume curve. The restriction flow-volume curve shows a FVC that is smaller than expected. The predicted volume is approximately 3 L; however, the patient's FVC is 2 L. In restriction, although the forced expiratory flows are often decreased, there is no concavity such as seen in the obstruction curve. It is important to note that spirometry does not diagnose restriction. The flow-volume loop will demonstrate findings such as this consistent with restriction; however, lung volumes are needed to define restriction. Concavity in the expiratory phase and a decreased FVC volume is consistent with a combination of an obstruction and restriction flow-volume curve.

mixed obstructive and restrictive disease by a combination of the two patterns.

Standardization of Spirometry Measurements

Spirometry is performed by having a person breathe into a tube (mouth piece) connected to a machine (spirometer) that measures the amount and flow of inhaled and exhaled air. Prior to performing spirometry, the appropriate technique is explained and demonstrated to the patient. Spirometry results are highly dependent on the completeness and speed of the patient's inhalation and exhalation, so the importance of completely filling and emptying the lungs of air during the test is emphasized. During spirometry, nose clips are worn to minimize air loss through the nose. The patient is seated comfortably without leaning or slumping and any restrictive clothing (such as ties or tight belts) are loosened or removed. The patient is instructed to take a full deep breath in and then blast the air out as quickly and forcefully as possible and to keep blowing the air out until all the air is exhaled. In general, the effort should last for a minimum of six seconds.

Like most medical tests, spirometry has seen changes over the years in equipment, computer support, and recommendations for standardization. In an effort to maximize the usefulness of spirometry results, the American Thoracic Society (ATS), in conjunction with the European Respiratory Society (ERS) developed standardizations of spirometry testing.[5,6] These recommendations are intended to decrease the variability of spirometry testing by improving the performance of the test. The recommendations cover equipment, quality control, training and education of people conducting the test, and training of patients performing the test. The recommendations also provide criteria for acceptability and reproducibility of the patient's spirometry efforts and guidelines on interpreting the spirometry test results. Because the results of spirometry depend on the patient's effort, at least three acceptable efforts are obtained with a goal of having the two highest measurements of FVC and FEV_1 vary <0.15 L.[6]

Other acceptability criteria include the following:

- Satisfactory start of test (no excessive hesitation or false start)
- No coughing during the first second of the effort
- No early termination of the effort
- No interruption in airflow (e.g., glottic closure)
- No evidence of a leak (mouth not tightly sealed around mouthpiece)
- No evidence of an obstructed mouthpiece (tongue, false teeth)

Bronchodilator Reversibility Test

One of many tests that may be useful in the diagnostic workup of asthma is spirometry with *reversibility.* Before the testing day, the patient is asked to (1) hold certain inhalers and medications that will influence the testing results, and (2) perform spirometry immediately before and 15–30 minutes after the administration of an inhaled, short-acting β2-adrenergic agonist. The ATS defines a positive bronchodilator response using changes in either the FEV_1 or the FVC using the percent increase and the increase in the volume exhaled after using a bronchodilator from baseline. A positive bronchodilator response is defined as an improvement of the FEV_1 and FVC by at least 12% and 200 mL from baseline.[7] The Global Initiative for Asthma defines airway

MINICASE 2

Bronchodilator Reversibility Study

Jessica T. is a 43-year-old female who presents to the pulmonary clinic with complaints of a dry cough at night three times a week, wheezing, and shortness of breath when exposed to pollen and cold air. She also has allergic rhinitis and eczema. She has never smoked tobacco.

		PREBRONCHODILATOR		POSTBRONCHODILATOR	
PFT	PREDICTED	MEASURED	% PREDICTED	MEASURED	% CHANGE
FVC (L)	3.11	2.45	78.83	2.65	8.1
FEV_1 (L)	2.56	1.53	59.82	2.02	32.02
FEV_1/FVC (%)		62.6		76.2	5.86

QUESTION: Do the results from this patient's spirometry test support the diagnosis of asthma?

DISCUSSION: The prebronchodilator FEV_1/FVC ratio is 62.6%, which is low for her predicted value and meets the definition of obstruction according to ATS/ERS guidelines. Her FEV_1 increased by more than 12% and 200 mL, which are the criteria for a positive bronchodilator test. The postbronchodilator FEV_1/FVC ratio normalizes to 76.2% (normal FEV_1/FVC is >75% for ages 40–59), which is also consistent with an asthma diagnosis. This test result along with the clinical suspicion for asthma based on respiratory triggers and history of atopy supports the diagnosis of asthma.

reversibility in adults as an increase in FEV_1 of at least 12% and 200 mL from baseline, with more confidence of a positive test with an increase in FEV_1 of at least 15% and 400 mL from baseline. For children, an increase of at least 12% of predicted for the FEV_1 is considered positive.[8] **Minicase 2** illustrates how bronchodilator reversibility study is useful for diagnosing asthma.

Peak Expiratory Flow Rate

The *peak expiratory flow rate* (PEFR), or peak flow, occurs within the first milliseconds of expiratory flow and is a measure of the maximum airflow rate. The PEFR can be measured with simple hand-held devices (peak flow meters) and is easily and inexpensively measured at a patient's home, in the clinician's office, or in the emergency department (ED). PEFR is less accurate than spirometry because it measures more of the upper airway over a shorter period of time, and readings can vary depending on the patient's efforts and meter type. PEFR is not the preferred method for determining airflow obstruction. Occasionally, PEFR is used to assess excessively variable airway obstruction triggered by certain exposures and to determine the severity of an asthma exacerbation.[8] During an acute exacerbation in the ED, patients are unable to complete spirometry, and PEFR is measured to determine the severity of the exacerbation and the efficacy of treatment.

Peak flow meters are designed for both pediatric and adult patients with PEFR between 60–400 L/min for children and between 60–850 L/min for adults. PEFR is measured by having a patient perform the following steps:

1. Stand up.
2. Move the indicator on the peak flow meter to the end nearest the mouthpiece.
3. Hold the meter and avoid blocking the movement of the indicator and the holes on the end of the meter.
4. Take a deep breath in and then seal mouth around the mouthpiece.
5. Blow out into the meter as hard and as fast as possible without coughing into the meter (like blowing out candles on a cake).
6. Examine the indicator on the meter to identify the number corresponding to the peak flow measurement.
7. Repeat the test two more times (remembering to move the indicator to the base of the meter each time).
8. Record the highest value of the three measurements in a diary (readings in the morning and afternoon are ideal).

To establish a patient's personal best peak flow rate, measure the peak flow rate over a two-week period of time when asthma symptoms and treatment are stable. The highest value over the two-week period of time is the personal best.

Body Plethysmography

Body plethysmography is a method used to obtain lung volume measures. Lung volume tests indicate the amount of gas contained in the lungs at the various stages of inflation. The lung volumes and capacities may be obtained by several methods, including body plethysmography, gas dilution, and imaging techniques.[9] Different methods can have small but significant effects on the values reported. Gas dilution methods only measure ventilated areas, whereas body plethysmography measures both ventilated and nonventilated areas. Therefore, body plethysmography values may be larger in patients with nonventilated or poorly ventilated lung areas. Computed tomography and magnetic resonance imaging can estimate lung volumes with additional detail of the lung tissue. As body plethysmography is the most commonly used method, this technique will be discussed in more detail.

In body plethysmography, a patient sits in an airtight box and is told to inhale and exhale against a closed shutter. Inside, a mouthpiece contains a pressure transducer. This is done to measure the change in pressure within the box during respiration. It senses the intrathoracic pressure generated when the patient rapidly and forcefully puffs against the closed mouthpiece. These data are then placed into the equation for Boyle's law:

$$P_1 \times V_1 = P_2 \times V_2$$

where P_1 = pressure inside the box where the patient is seated (atmospheric pressure); V_1 = volume of the box; P_2 = intrathoracic pressure generated by the patient; and V_2 = calculated volume of the patient's thoracic cavity. Because temperature (T_1 and T_2) is constant throughout testing, it is not included in the calculations.

By applying Boyle's law, this test will provide a measure of the functional residual capacity (FRC) or the volume of gas remaining at the end of a normal breath. Once the FRC is determined, the other lung volumes and capacities can be calculated based on this FRC and volumes obtained in static spirometry. After these data are generated, the patient's plethysmography results are usually compared to references from a presumed normal population. This comparison necessitates the generation of predicted values for that patient if he or she were completely normal and healthy. Through complex mathematical formulas, sitting and standing height, age, sex, race, barometric pressure, and altitude are factored in to give predicted values for the pulmonary functions being assessed.[6] The patient's results are compared to the percentage of predicted values based on the results of these calculations.

Lung Volumes and Lung Capacities

Lung volumes include tidal volume (TV), inspiratory reserve volume (IRV), expiratory reserve volume (ERV), and residual volume (RV). These four volumes in various combinations make up *lung capacities*, which include inspiratory capacity (IC), FRC, SVC, and total lung capacity (TLC).

TV, IRV, ERV, and RV

The *TV* is the amount of air inhaled and exhaled at rest in a normal breath. It is usually a very small proportion of the lung volume and is infrequently used as a measure of respiratory

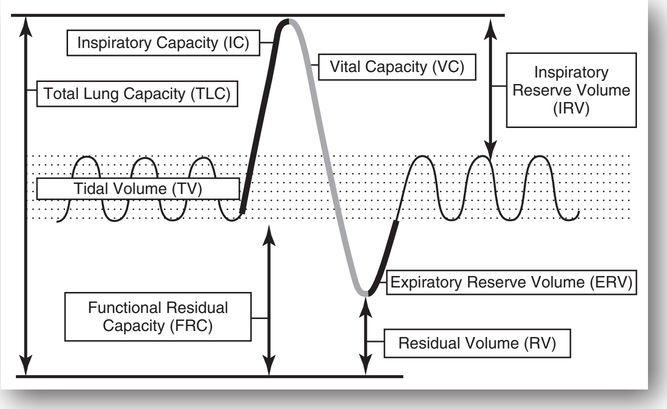
Inspiratory Capacity (IC)
Total Lung Capacity (TLC)
Vital Capacity (VC)
Inspiratory Reserve Volume (IRV)
Tidal Volume (TV)
Expiratory Reserve Volume (ERV)
Functional Residual Capacity (FRC)
Residual Volume (RV)

FIGURE 14-3. Lung volumes and capacities—a schematic representation of various lung compartments based on a typical spirogram. See reference 6 for more information.

disease. The IRV is the volume measured from the "top" of the TV (i.e., initial point of normal exhalation) to the maximal inspiration. During exhalation, the ERV is the volume from the "bottom" of the TV (i.e., initial point of normal inhalation) to maximal expiration. The RV is the volume of air left in the lungs at the end of forced expiration to the bottom of ERV. Without the RV, the lungs would collapse like deflated balloons. In diseases characterized by obstructions that trap air in the lungs (e.g., COPD), the RV increases, and the patient is less able to mobilize air trapped behind these obstructions. These four lung volumes are depicted graphically in **Figure 14-3**.

IC, FRC, SVC, and TLC

The *IC* is the volume measured from the point of the TV where inhalation normally begins to maximal inspiration, and it is a summation of TV and IRV. The *FRC* is the sum of the ERV and RV, and it is the volume of gas remaining in the lungs at the end of the TV. It also may be defined as a balance point between chest wall forces that increase volume and lung parenchymal forces that decrease volume. An increased FRC represents hyperinflation of the lungs and usually indicates airway obstruction. The FRC may be decreased in diseases that affect many alveoli (e.g., pneumonia) or by restrictive changes, especially those due to fibrotic pulmonary tissue changes. The *SVC* is the volume of air that is exhaled as much as possible after inhaling as much as possible. It is a summation of the IRV, TV, and ERV and was described in more detail under spirometry measurements. The *TLC* is the summation of all four lung volumes (IRV + TV + ERV + RV). It is the total amount of gas contained in the lungs at maximal inhalation.

Diffusion Capacity Tests

Tests of gas exchange measure the ability of gases to cross (diffuse) the alveolar-capillary membrane and are useful in assessing interstitial lung disease.[10] Typically, these tests measure the per minute transfer of a gas, usually carbon monoxide (CO), from the alveoli to the blood. CO is used because it is a gas that is not normally present in the lung, has a high affinity for Hgb in red blood cells, and is easily delivered and measured. The diffusion capacity may be lessened following losses in the surface area of the alveoli or thickening of the alveolar-capillary membrane. Membrane thickening may be due to infiltration of inflammatory cells or fibrotic changes. These test results can be confounded by a loss of diffusion capacity due to poor ventilation, which may be related to closed or partially closed airways (as with airway obstruction) or to a ventilation-perfusion mismatch (as with pulmonary emboli or pulmonary hypertension). The diffusion capacity of the lungs to CO can be measured by either a single-breath test or steady-state test.

In the single-breath test, the patient deeply inhales—to VC—a mixture of 0.3% CO, 10% helium, and balance air. After holding his or her breath for 10 seconds, the patient exhales fully, and the concentrations of CO and helium are measured during the end of expiration (i.e., alveolar flow). These concentrations are compared to the inspired concentrations to determine the amount diffusing across the alveolar membrane. The mean value for CO is about 25–30 mL/min/mm Hg.

In the steady-state test, the patient breathes a 0.1–0.2% concentration of CO for five to six minutes. In the final two minutes, the expired gases are collected and ABGs are obtained. The exhaled gas is measured for total volume and concentrations of CO, carbon dioxide, and oxygen. The ABG is analyzed for carbon dioxide. These values are used to calculate the amount of gas transferred across the alveolar membrane per unit of time (usually a minute). The usual mean value may be slightly less with the steady-state method than the single-breath method. Furthermore, females typically have slightly lower values than males, probably due in part to slightly smaller lung volumes.

These diffusion capacity test are useful for assessing gas exchange. A normal DLCO is considered ≥70% of the predicted value for the patient. Diffusion capacity is decreased in diseases that cause alveolar fibrotic changes. Changes may be idiopathic, such as those seen with sarcoidosis or environmental or occupational disease (asbestosis and silicosis), or be induced by drugs (e.g., nitrofurantoin, amiodarone, and bleomycin).[11,12] Anything that alters Hgb, decreases the red blood cell Hgb concentration, or changes diffusion across the red blood cell membrane may alter the DLCO. The DLCO also reflects the pulmonary capillary blood volume. An increase in this volume (pulmonary edema or asthma) may increase the DLCO. **Minicase 3** describes how pulmonary function tests are used to diagnose restrictive airway disease.

Specialized Tests

Bronchial Provocation Tests

Bronchial provocation tests (BPTs) are used to (1) aid in the diagnosis of asthma when the more common tests (symptom history, spirometry with reversibility) cannot confirm or reject the diagnosis; (2) evaluate the effects of drug therapy on airway hyperreactivity; and (3) evaluate potential drug effectiveness. A BPT measures the reactivity of the airways to known

MINICASE 3

Using Pulmonary Function Tests to Evaluate a Patient with Interstitial Lung Disease

Jacob K. is a 55-year-old male who presents to the medicine clinic with complaints of progressive dyspnea on exertion and minimal dry cough for the past three months. He has a history of rheumatoid arthritis and was started on methotrexate four months ago. The CT of the chest shows diffuse ground glass opacities consistent with active inflammation and some minimal fibrosis at the bases. He had a PFT performed over a year ago that was completely normal. A repeat PFT is ordered and includes spirometry, lung volumes, and diffusion capacity. His PFT reveals the following results and the flow-volume curve in Figure 14-2 labeled Restriction.

QUESTION: How are these PFTs useful in the diagnosis, evaluation, and management of this patient?

DISCUSSION: Looking at his PFT in the table below, the FVC is 63.6% of predicted (reduced), the FEV$_1$ is 61.6% of predicted (reduced), and the FEV$_1$/FVC ratio is 82.2% (normal). This is consistent with a restrictive pattern. A TLC is 55.58% of predicted (reduced), verifying a restrictive pulmonary defect, and the DLCO is only 29% of predicted (normal range is ≥70% of predicted). These findings are helpful in the diagnosis of interstitial lung disease in the setting of an abnormal CT scan and change from previous normal spirometry. The severity of restriction can also be determined by the amount of decrease in TLC.

	PREBRONCHODILATOR		POSTBRONCHODILATOR		
PFT	**PREDICTED**	**MEASURED**	**% PREDICTED**	**MEASURED**	**% CHANGE**
FVC	3.09	1.97	63.58	1.87	−4.99
FEV$_1$	2.62	1.62	61.60	1.53	−5.45
FEV$_1$/FVC %		82.20		81.80	−0.49
TLC	4.57	2.54	55.58		
VC	3.09	1.83	59.09		
RV	1.50	0.71	47.74		
FRC	2.56	1.64	64.00		
DLCO	22.95	6.66	29.01		

DCLO = carbon monoxide diffusion capacity; FEV$_1$ = forced expiratory volume in one second; FRC = functional residual capacity; FVC = forced vital capacity; TLC = total lung capacity; RV = residual volume; VC = vital capacity.

He is diagnosed with methotrexate-induced lung disease. The methotrexate is discontinued, and he is treated with prednisone. After three months of therapy, a repeat PFT is performed. The FVC is 75% of predicted, the FEV$_1$ is 72% of predicted, and the FEV$_1$/FVC ratio is 80%. The TLC has increased to 65%, and the DLCO has increased to 40% of predicted. The repeat PFT shows that he is responding to pharmacotherapy. He reports improvement in his symptoms. The follow-up PFT is used to help evaluate the response to discontinuing the offending medication and to establish a new pulmonary function status.

concentrations of agents that induce airway narrowing. Negative BPTs are useful in excluding the diagnosis of asthma more than confirming the diagnosis when a test is positive. Using this technique in research, the magnitude and duration of the effect of different drugs on the airways may be compared. BPTs are often referred to as *challenges,* as the airways are challenged with increasing doses of a provoking agent until a desired drop (usually 20%) in lung function occurs. Agents used to provoke the lung include inhaled methacholine, histamine, adenosine, and specific allergens.[13,14] The ATS has published guidelines for methacholine and exercise challenge testing to enhance the safety, accuracy, and validity of the tests.[14]

Bronchial provocation testing begins by measuring baseline spirometry parameters to ensure it is safe to conduct the test. A BPT should not be performed if the FEV$_1$ is <60% of predicted.[14] Most BPTs then begin with nebulization of a solution of phosphate buffered saline. This both serves as a placebo to assess the airway effect of nebulization and establishes baseline airway function from which the amount of pulmonary function to be reduced is calculated. Then an extremely low concentration of the selected bronchoconstrictor agent is nebulized followed by spirometry at 30 and 90 minutes from the end of the nebulization. Additional spirometry efforts may be done to meet or exceed ATS criteria. Optimally, the FEV$_1$ values should be within 0.10 L of each other.[14] The patient inhales gradually increasing concentrations of the bronchoconstrictor at specific time intervals (usually five minutes) until a predesignated amount of airway restriction is attained. This is usually defined as a reduction in FEV$_1$ by at least 20% from the saline control. The challenge data are then summarized into a single number, the PC$_{20}$FEV$_1$ (mg/mL). This refers to the provocation concentration of the bronchoconstriction agent that would produce a 20% reduction in FEV_1.

For methacholine, a $PC_{20}FEV_1$ of <1 mg/mL indicates moderate-to-severe bronchial hyperresponsiveness (BHR), 1–4 mg/mL as mild BHR, 4–16 mg/mL as borderline BHR, and >16 mg/mL as normal BHR. During a BPT, patients may experience transient respiratory symptoms such as cough, shortness of breath, wheezing, and chest tightness. An inhaled, short-acting β2-adrenergic agonist or anticholinergic agent may be administered to alleviate symptoms and quicken the return of the FEV_1 to the baseline value. Because BPTs can elicit severe, life-threatening bronchospasm, trained personnel and medications to treat severe bronchospasm should be on hand in the testing area.

Exercise Challenge Testing

Exercise challenge testing is used to confirm or rule out exercise-induced bronchospasm (EIB) and to evaluate the effectiveness of medications used to treat or prevent EIB, which occurs usually in patients with normal PFTs who become symptomatic with exercise. The etiology of EIB is thought to be related to the cooling and drying of the airways caused by rapid breathing during exercise.

Exercise tests are usually done with a motor driven treadmill (with adjustable speed and grade) or an electromagnetically braked cycle ergometer. Heart rate should be monitored throughout the test. Nose clips should be worn, and the room air should be dry and cool to promote water loss from the airways during the exercise test. In most patients, symptoms are effectively blocked by use of an inhaled bronchodilator immediately prior to beginning exercise or other exertion causing the problem. After obtaining baseline spirometry, the exercise test is started at a low speed that is gradually increased over two to four minutes until the heart rate is 80–90% of the predicted maximum or the work rate is at 100%. The duration of the exercise is age and tolerance dependent. Children <12 years of age generally take six minutes, while older children and adults take eight minutes to complete the test. After the exercise is completed, the patient does serial spirometry at five-minute intervals for 20–30 minutes. FEV_1 is the primary outcome variable. A 10% or more decrease in FEV_1 from baseline is generally accepted as an abnormal response, although some clinicians feel a 15% decrease is more diagnostic of EIB.[15]

Six-minute walk test. The *six-minute walk test* (6MWT) is a test used to measure the distance a patient can walk on a flat, hard surface in sixminutes.[16] The results of the test are used to determine if a patient requires continuous oxygen at home. The results have also been correlated to the patient's quality of life and abilities to complete daily activities. The results of the 6MWT also help predict morbidity and mortality for patients with congestive heart failure, COPD, and primary pulmonary hypertension.[17-20] Pulmonary hypertension studies use this test to monitor the efficacy of interventions with medications.[20]

While performing the 6MWT, the patient is educated that the goal of the test is to walk as far as possible in six minutes, allowing the patient to select the intensity of exercise. Stopping and resting is allowed during the test. Pulse oximetry is optional during the test but is often used. Reference equations for healthy adults have been published and normal values in young healthy children have been studied.[21-23] Normal values in healthy adults range from 500–630 meters and healthy children 4–11 years of age had a mean distance walked of 470 meters (±59 meters). Clinical improvement has been correlated with an increase in distance of 54 meters; alternatively, percent change from baseline may be calculated.[24] Practice tests, younger age, taller height, less weight, male, longer corridor length, and encouragement all improve test results. Unstable angina and myocardial infarction in the past month, a resting heart rate >120 beats per minute, or a blood pressure reading >180/100 are all contraindications for performing the 6MWT. In practice, the 6MWT is also used to assess the amount of oxygen needed with exertion. Patients with mild-to-moderate pulmonary disease may have normal oxygen saturation at rest but poor saturation with exertion. An oxygen saturation of 88% or lower indicates the need for supplemental oxygen.

Spirometry and the Pediatric Population

Children two to six years of age are able to perform spirometry with specific equipment and personnel trained to work with this population.[25] Although clinical application remains debatable, spirometry for this age population is becoming more prominent in clinical research. Adult criterion for testing is not applicable to this age group. For instance, at this age full exhalation is complete prior to one second, and therefore the $FEV_{0.5}$, (forced expiratory volume in 0.5 second) may be more appropriate to measure than the FEV_1. Repeatability of two flow curves for the FVC and FEV_1 is defined as two measurements within 0.1 L or 10% of each other. Flexibility because of poor repeatability is recommended with a young child. Reference data equations for this age group have not been endorsed by ATS or ERS.

Carbon Monoxide Breath Test

CO is a poisonous gas emitted from anything burning, including cigarette smoke. As mentioned under the DLCO section, CO binds more readily to Hgb than oxygen, causing increased fatigue and shortness of breath. With a simple breath test by a handheld CO meter, the patient can see how much CO is in the body (parts per million [ppm]) and in the blood (% COHgb). In clinical studies, 10 ppm or less is often defined as a nonsmoker; however, in clinical practice, depending on the meter utilized, the level is usually lower. This objective reading is thought to be a motivator for some to quit smoking and remain abstinent.[26] However, a Cochrane review found no significant increase in abstinence rates with CO measurements.[27] Calculating lung age using the measured FEV_1 and discussing this with the smoker was found to be a motivator for smokers to quit smoking in a clinical trial and may be applicable to clinical practice.[28]

Testing exhaled CO is a simple breath test where the patient holds his or her breath for 15 seconds then exhales into a meter. The meter is able to indicate how much CO is in the patient's lungs and estimate how much is attached to Hgb in the patient's blood. The test is an objective value that patients can visually see the effects of inhaling smoke with higher values of CO detected. After 8–12 hours without smoking, CO levels become undetectable.

Fractional Exhaled Nitric Oxide

Measurement of exhaled concentrations of nitric oxide (NO) is a noninvasive biomarker test of airway inflammation for both diagnosing and monitoring eosinophilic airway inflammation.[29] Various handheld devices using the patient's breath are available that include an electrochemical sensor to determine the exhaled nitric oxide concentration. *Fractional exhaled nitric oxide* (FENO) results >50 parts per billion (ppb) in adults and >35 ppb in children younger than 12 years of age indicate eosinophilic inflammation with a high likelihood to respond to corticosteroids.[30] As a monitoring test, FENO results are best interpreted as changes from baseline for each patient rather than using population normal readings. FENO measurements have many confounding factors including smoking history, age, and sex. The test should be used in context with the patient history and as a tool with other diagnostic and monitoring tests.

SUMMARY

This chapter discusses the importance of pulmonary function testing as it relates to the diagnosis, treatment, and monitoring of respiratory disease states. After a review of the anatomy and physiology of the lungs, the mechanics of obtaining PFTs were emphasized. By understanding these mechanics, a clinician can better understand the interpretation of PFTs, use findings from different PFTs to help differentiate among diagnoses, and assist in making optimal therapeutic recommendations. PFT results are not interpreted in isolation, but they need to be assessed within the context of the other findings from the medical history and from other laboratory or clinical test results.

Common tests of airflows and lung volumes are primarily used to characterize airway functions and diseases. These measurements may indicate the need for specific medication interventions. Other tests, such as diffusion capacity, indicate the ability of a gas to diffuse through lung tissues and the general thickness of the membranes lining the alveoli. Specialized tests, such as bronchial provocation and bronchodilator reversibility tests, are used to guide treatment choices. Clearly, the PFTs are an important tool to aid the clinician in decision making.

LEARNING POINTS

1. ***What is a PFT?***

   ANSWER: A PFT is an assessment of lung function that is composed of several different components (e.g., spirometry, volumes, diffusion capacity). The component of the PFT to be ordered is determined by the information needed. For example, spirometry is performed to reveal the presence of obstructive disease. Lung volumes determine the presence of restrictive disease, and the diffusion capacity test ascertains the adequacy of gas exchange.

2. ***Why is spirometry an important test in the diagnosis of COPD?***

   ANSWER: In COPD, postbronchodilator spirometry is necessary to determine the presence of persistent airflow obstruction and the degree of disease severity. The test is also important to rule out other causes of shortness of breath and cough, like heart failure. Physical exam and history alone are often not adequate to detect airway obstruction. Therefore, an objective test with spirometry is needed to confirm a clinical suspicion. Based on GOLD guidelines, the ATS/ERS guidelines medical management of COPD is based in part on spirometry results, which also will often guide therapy.

3. ***Does a significant bronchodilator response on spirometry testing differentiate asthma from COPD?***

   ANSWER: Traditionally, reversibility after bronchodilator use was considered a criterion to differentiate asthma and COPD. However, evidence has shown that a significant bronchodilator response is common in COPD as well and this assessment is no longer used to differentiate between asthma and COPD. It is still a useful test when used in conjunction with a clinical history (e.g. risk factors for COPD, presentation of shortness of breath, evidence of atopy), physical exam, and other PFTs to support a clinical suspicion of asthma and COPD.

4. ***How is restrictive lung disease diagnosed?***

   ANSWER: It is important to note that spirometry only can provide evidence consistent with restrictive disease such as a decrease in FEV_1 and FVC with a normal or elevated FEV_1/FVC ratio. However, restriction is a decrease in lung volume as defined by a decrease in the TLC, which is obtained by lung volume tests, such as body plethysmography. Test results are needed to diagnose restrictive lung disease.

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15

LIVER AND GASTROENTEROLOGY TESTS

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OBJECTIVES

After completing this chapter, the reader should be able to

- Discuss how the anatomy and physiology of the liver and pancreas affect interpretation of pertinent laboratory test results
- Classify liver test abnormalities into cholestatic and hepatocellular patterns and understand the approach to evaluating patients with these abnormalities
- Explain how hepatic and other diseases, as well as drugs and analytical interferences, cause abnormal laboratory test results for bilirubin
- Understand hepatic encephalopathy and the role of serum ammonia in its diagnosis
- Discuss the laboratory test abnormalities typically associated with hemochromatosis
- Design and interpret a panel of laboratory studies to determine if a patient has active, latent, or previous viral hepatitis infection
- Understand the significance and utility of amylase and lipase in evaluating abdominal pain and pancreatic disorders
- Discuss the role of *Helicobacter pylori* in peptic ulcer disease and the tests used to diagnose it
- Discuss the tests and procedures used to diagnose *Clostridium difficile* colitis

Hepatic and other gastrointestinal (GI) abnormalities can cause a variety of clinically significant diseases, in part because of their central role in the body's biochemistry. This chapter provides an introduction to common laboratory studies used to investigate these diseases. Studies of the liver are roughly divided into those associated with (1) synthetic liver function, (2) excretory liver function and cholestasis, (3) hepatocellular injury, and (4) detoxifying liver function and serum ammonia. Specific tests may also be used to investigate specific disease processes including viral hepatitis, primary biliary cirrhosis (PBC), and hemochromatosis. This chapter also covers several tests for specific nonhepatic disease processes (including pancreatitis, *Helicobacter pylori* [*H. pylori*] infection, and *Clostridium difficile* [*C. difficile*] colitis).

ANATOMY AND PHYSIOLOGY OF THE LIVER AND PANCREAS

Liver

The *liver,* located in the right upper quadrant of the abdomen, is the largest solid organ in the human body.[1] It has two sources of blood:

1. The hepatic artery, originating from the aorta, supplies arterial blood rich in oxygen.
2. Portal veins shunt the venous blood from the intestines to the liver. This transports absorbed toxins, drugs, and nutrients directly to the liver for metabolism.

The liver is divided into thousands of lobules (**Figure 15-1**). Each lobule is comprised of plates of hepatocytes (liver cells) that radiate from the central vein much like spokes in a wheel. Between adjacent liver cells formed by matching grooves in the cell membranes are small bile canaliculi. The hepatocytes continually form and secrete bile into these canaliculi, which empty into terminal bile ducts. Subsequently, like tiny streams forming a river, these bile ducts empty into larger and larger ducts until they ultimately merge into the common duct. Bile then drains into either the gallbladder for temporary storage or directly into the duodenum.

The liver is a complex organ with a prominent role in all aspects of the body's biochemistry. It takes up amino acids absorbed by the intestines, processes them, and synthesizes them into circulating proteins including albumin and clotting factors. The liver is also involved in the breakdown of excess amino acids and processing of byproducts including ammonia and urea. The liver plays a similar role in absorbing carbohydrates from the gut, storing them in the form of glycogen, and releasing them as needed to prevent hypoglycemia. Most lipid and lipoprotein metabolism, including cholesterol synthesis, occurs in the liver. The liver is the primary location for detoxification and excretion of a wide variety of endogenous substances produced by the body (including sex hormones) as well as exogenous substances absorbed by the intestines (including a panoply of drugs and toxins). Thus, in patients with liver failure, standard dosing of some medications can lead to dangerously high serum concentrations and toxicity. The role of the liver in bilirubin metabolism is explored further below.

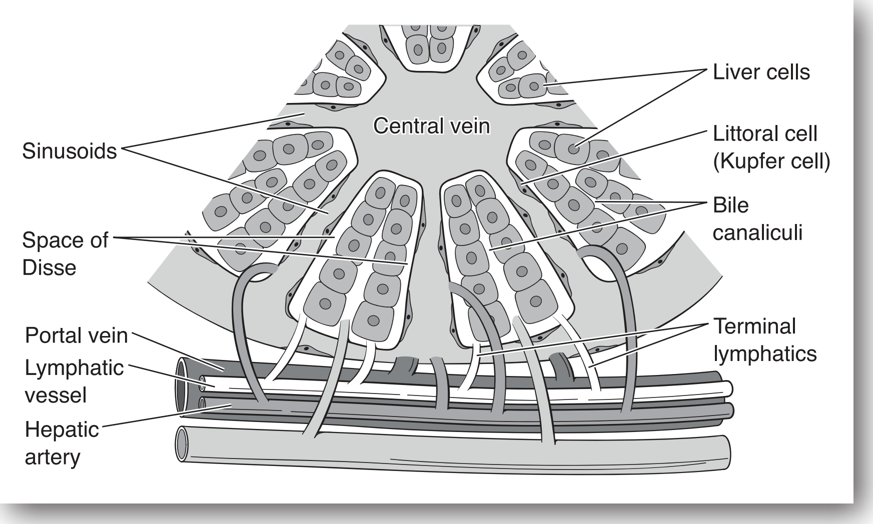


FIGURE 15-1. Basic structure of a liver lobule including the lymph flow system comprised of the spaces of Disse and interlobular lymphatics. *Source:* Reproduced with permission from Guyton AC. Medical Physiology. 5th ed. Philadelphia: WB Saunders; 1976.

With its double blood supply, large size, and critical role in regulating body metabolic pathways, the liver is affected by many systemic diseases. Although numerous illnesses affect the liver, it has tremendous reserve capacity and can often maintain its function in spite of significant disease. Furthermore, the liver is one of the few human organs capable of regeneration.

Pancreas

The *pancreas* is an elongated gland located in the retroperitoneum. Its head lies in close proximity to the duodenum, and the pancreatic ducts empty into the duodenum. The pancreas has both exocrine glands (which secrete digestive enzymes into the duodenum) and endocrine glands (which secrete hormones directly into the circulation).

The pancreatic exocrine glands produce enzymes that aid in digestion of proteins, fats, and carbohydrates (including trypsin, chymotrypsin, lipase, and amylase). Insufficient enzyme production (i.e., pancreatic exocrine insufficiency) is associated with malabsorption of nutrients, leading to progressive weight loss and severe diarrhea. The glands also produce many hormones including insulin and glucagon. Insufficient insulin production leads to diabetes mellitus. Thus, the pancreas plays an important role in digestion and absorption of food as well as metabolism of sugar. Like the liver, the pancreas has a tremendous reserve capacity; over 90% glandular destruction is required before diabetes or pancreatic insufficiency develops.

AN INTRODUCTION TO LIVER TESTS AND THE LFT PANEL

Investigation of liver disease often begins with obtaining a panel of liver tests generally referred to as the *LFT panel* or *LFTs* (liver function tests). This panel may vary slightly between hospitals and laboratories but generally includes the aminotransferases (previously referred to as *transaminases*), including aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin, alkaline phosphatase (ALP), and albumin. LFT is a misnomer because not all of these tests actually measure liver function (specifically, aminotransferases reflect liver injury). Additionally, the liver has several functions, and different tests reflect these different functions. **Table 15-1** divides liver tests into rough categories. Although there is considerable overlap between these categories, these divisions may provide an initial framework for understanding the LFT panel.

This grouping of tests mirrors a division of liver diseases into two broad categories: cholestatic and hepatocellular. In cholestatic disease, there is an abnormality in the excretory function of the liver (i.e., namely secretion of bile by hepatocytes and passage of bile through the liver and bile ducts into the

TABLE 15-1. Categories of Liver Tests

PROCESS	MOST CLOSELY RELATED TESTS
Protein synthesis	Albumin Prealbumin PT/INR (clotting factors)
Excretion into the bile ducts and drainage into the duodenum (impairment of this process is defined as cholestasis)	Bilirubin ALP 5'-nucleotidase GGT
Hepatocellular injury	Aminotransferases: AST ALT
Detoxification	Ammonia (NH_3+)

ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; GGT = gamma-glutamyl transpeptidase; INR = international normalized ratio; PT = prothrombin time.

duodenum). In hepatocellular disease, there is primary inflammation and damage to the hepatocytes themselves (e.g., due to viral infection of the hepatocytes). These two categories may overlap because disease of the hepatocytes (hepatocellular processes), if severe enough, will also lead to derangement of bile secretion. However, the distinction between primarily cholestatic versus primarily hepatocellular diseases and, in turn, LFT patterns remains useful and fundamental.[2] Further confusing is that liver tests may be abnormal in patients with diseases that do not affect the liver.

The range of normal laboratory values used here is taken from *Harrison's Principles of Internal Medicine*, 19th edition, and *Nelson's Textbook of Pediatrics*, 20th edition.[3] Reference ranges may vary slightly between different laboratories, and most laboratories will list their reference ranges along with laboratory results. Listed normal ranges are for adult patients, and pediatric patients will often have different ranges of normal values.

TESTS OF SYNTHETIC LIVER FUNCTION

As discussed above, one of the functions of the liver is to synthesize proteins that circulate in the blood, including albumin and clotting proteins. Measurement of the levels of these proteins in the blood provides a reflection of the ability of the liver to synthesize them. The liver has an enormous reserve function, so that it may synthesize normal amounts of proteins despite significant liver damage. Therefore, tests of synthetic function are not sensitive to low levels of liver damage or dysfunction. Inadequate protein synthetic function is mainly limited to hepatic cirrhosis, scarring of the liver that can result from years of alcohol abuse, inflammation, or massive liver damage (e.g., due to alcoholic liver disease, severe acute viral hepatitis, unrecognized and untreated chronic hepatitis, or potentially lethal toxin ingestion). In these situations, measuring synthetic function may be useful in determining prognosis by reflecting the degree of hepatic failure. The most commonly used tests of protein synthetic function are albumin, prothrombin time (PT), and international normalized ratio (INR).

Albumin

Normal range[3]: 4–5 g/dL (40–50 g/L)

Albumin is a major plasma protein that is involved in maintaining plasma oncotic pressure and the binding and transport of numerous hormones, anions, drugs, and fatty acids.[4] The normal serum half-life of albumin is about 20 days, with about 4% degraded daily.[5] Because of albumin's long half-life, serum albumin measurements are slow to fall after the onset of hepatic dysfunction (e.g., complete cessation of albumin production results in only a 25% decrease in serum concentrations after eight days).[1] For this reason, levels are often normal in acute viral hepatitis or drug-related hepatotoxicity.[2] Alternatively, albumin is commonly reduced in patients with chronic synthetic dysfunction due to cirrhosis.

Albumin levels may be low due to a variety of other abnormalities in protein synthesis, distribution, and excretion in addition to liver dysfunction. These include malnutrition/malabsorption, protein loss from the gut, kidney, or skin (as in nephrotic syndrome, protein-losing enteropathy, or severe burns, respectively), or increased blood volume (e.g., following administration of large volumes of intravenous [IV] fluids).[6-9] Albumin is a negative acute phase reactant, meaning that in the setting of systemic inflammation (e.g., as due to infection or malignancy), the liver will produce less albumin. Severely ill, hospitalized patients commonly have low albumin levels due to a combination of poor nutrition, systemic inflammation, and IV fluid administration. In these patients, extremely low albumin concentrations carry a poor prognosis irrespective of any particular liver disease. Given the numerous causes of a low albumin level, it is important to interpret it within the context of each patient. For example, in a patient with metastatic cancer and no known liver disease, a low albumin level suggests decreased nutritional intake and advanced malignancy with systemic inflammation. Alternatively, in a patient with known cirrhosis, a low albumin level suggests severe chronic liver failure. In a patient with no known medical disease, a low albumin level suggests the presence of significant disease and requires further investigation.

Hypoalbuminemia itself is usually not associated with specific symptoms or findings until concentrations become quite low. At very low concentrations (<2–2.5 g/dL), patients can develop peripheral edema, ascites, or pulmonary edema. Albumin normally generates oncotic pressure, which holds fluid in the vasculature. Under conditions of low albumin, fluid leaks from the vasculature into the interstitial spaces of subcutaneous tissues or into the body cavities. Low albumin concentrations increase the concentration of ionized (nonprotein bound, physiologically active) calcium in the bloodstream. This must be taken into account when interpreting the total serum calcium level. Finally, in the presence of low albumin concentration, the percentage of nonprotein bound medication in the bloodstream is increased for highly protein bound agents (e.g., phenytoin, warfarin, and salicylates). This could result in increased pharmacologic effects or adverse effects from usual doses of the medications.

Hyperalbuminemia is seen in patients with marked dehydration (which concentrates their plasma), where it is associated with concurrent elevations in blood urea nitrogen (BUN) and hematocrit. Patients taking anabolic steroids may demonstrate truly increased albumin concentrations, but those on heparin or ampicillin may have falsely elevated results with some assays. Hyperalbuminemia is asymptomatic.

Prealbumin (Transthyretin)

Normal range[3]: 17–34 mg/dL (170–340 mg/L)

Prealbumin is similar to albumin in several respects: it is synthesized primarily by the liver; it is involved in the binding and transport of various solutes (thyroxin and retinol); and it is affected by similar factors that affect albumin levels.

The primary difference between the two proteins is that prealbumin has a short half-life (2 days, compared to 20 days for albumin) and a smaller body pool than albumin, making the former more rapidly responsive than albumin.[10] Additionally, due to its high percentage of tryptophan and essential amino acids, prealbumin is more sensitive to protein nutrition than albumin and is less affected by liver disease or hydration state than albumin.[11] In practice, prealbumin is generally used to assess protein calorie nutrition. Prealbumin is generally regarded as the best laboratory test of protein nutrition, and it is routinely used to monitor patients receiving total parenteral nutrition (TPN) or tube feeding.[12-14]

International Normalized Ratio and Prothrombin Time

Normal range: INR 0.9–1.1, PT 12.7–15.4 sec

For an introduction to *international normalized ratio* (INR) and *prothrombin time* (PT), please see Chapter 17. These two tests measure the speed of a set of reactions in the extrinsic pathway of the coagulation cascade. Decreased synthesis or impaired activation of clotting factors correlates with prolonged reaction times, and increased values of INR and PT. Both PT and INR are two different measures of the same set of reactions, with the INR being a derived index that takes into account variations between test reagents used in different laboratories. As such, INR is more precise and easily interpretable, and replaces the use of the PT.

The liver is required for the synthesis of clotting factors (with the exception of factor VIII), many of which require a vitamin K cofactor for their activation. Therefore, either hepatic impairment or vitamin K deficiency may lead to a deficiency in activated clotting factors with subsequent prolongation of PT/INR. Both synthetic failure and vitamin K deficiency may also cause prolongation of activated partial thromboplastin time, which measures a different set of coagulation reactions in the intrinsic coagulation cascade) but to a much lesser degree than PT/INR.

The prolongation of PT/INR alone is not specific for liver disease. It can be seen in many situations, most of which interfere with the utilization of vitamin K, a cofactor required for the proper posttranslational activation of clotting factors II, VII, IX, and X. Because vitamin K is a fat soluble vitamin, inadequate vitamin K in the diet or fat malabsorption as caused by cholestasis may cause hypovitaminosis.[15] Many broad-spectrum antibiotics, including tetracyclines, may eliminate vitamin K–producing flora in the gut. The anticoagulant agent warfarin interferes directly with vitamin K dependent activation of clotting factors.

If the etiology of elevated PT/INR remains unclear despite obtaining additional coagulation tests, then the clinical approach is to provide parenteral vitamin K.[16] Although commonly given as a subcutaneous injection, vitamin K (10 mg) can be given by slow IV infusion in patients with prolonged PT/INR with serious bleeding. If the PT/INR is prolonged due to malabsorption, warfarin, perturbed gut flora, or the absence of vitamin K in the diet, the PT/INR usually corrects by at least 30% within 24 hours.[9] Alternatively failure of PT/INR to normalize despite parenteral vitamin K suggests impaired synthetic liver function (**Figure 15-2**).[4] Other factors that may cause a prolonged PT/INR, which does not respond to parenteral vitamin K, include inherited clotting factor deficiencies.

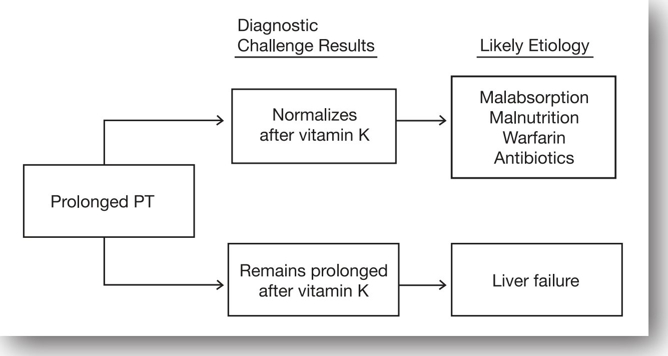

Diagnostic Challenge Results
Likely Etiology
Prolonged PT
Normalizes after vitamin K
Malabsorption
Malnutrition
Warfarin
Antibiotics
Remains prolonged after vitamin K
Liver failure

FIGURE 15-2. Evaluation of a prolonged PT/INR.

Because clotting factors are produced in excess of need and because the liver has tremendous synthetic reserves, only substantial hepatic impairment (>80% loss of synthetic capability) leads to decreased synthesis of these factors and subsequent clotting abnormalities.[6] Thus, PT/INR, albumin, and prealbumin levels lack sensitivity and may remain normal in the face of substantial liver damage.[4] However, they have considerable prognostic value if liver damage is sufficient to affect them. Unlike albumin (which responds slowly to hepatic insult), PT/INR responds within 24 hours to changes in hepatic status due to the short half-life of certain clotting proteins (i.e., factor VII has a half-life of less than six hours).[17] Thus, the PT/INR may become elevated days before other manifestations of liver failure and, likewise, may normalize prior to other evidence of clinical improvement.[18] One utility of determining PT/INR in liver disease is to provide prognostic data, generally in situations where the cause of the elevated PT/INR is known, for example, acute acetaminophen overdose leading to hepatic failure.

In addition to serving as an LFT, PT/INR has direct clinical relevance in accessing the patient's tendency to bleed spontaneously, or due to surgical or diagnostic procedures. Bleeding is a dramatic complication of hepatic failure. When the PT/INR is significantly elevated, bleeding may be controlled or at least diminished by fresh frozen plasma, which contains the needed activated clotting factors and often corrects the PT/INR temporarily.

Des-gamma-carboxy prothrombin (DCP) is an abnormal prothrombin form that is released in the absence of vitamin K, in the presence of vitamin K antagonists (warfarin), and by certain tumors (hepatocellular carcinoma). Although it has been used to evaluate the risk of hepatocellular carcinoma, DCP has a lower sensitivity than α-fetoprotein for tumors under 3 cm. in size.[19]

CHOLESTATIC LIVER DISEASE

Cholestasis is a deficiency of the excretory function of the liver. As described above, bile is normally secreted by hepatocytes into bile canaliculi, where it flows into larger bile ducts, and eventually empties into the duodenum. Excretion of bile from the liver serves multiple purposes. Certain large lipophilic toxins, drugs, and endogenous substances are eliminated by secretion into the bile with eventual elimination in the feces. Bile salts also play an important role in dissolving and absorbing dietary fat-soluble vitamins and nutrients within the small intestine.

Failure of the excretory functions of the liver leads to a predictable set of consequences. Substances normally secreted in the bile accumulate, resulting in jaundice (from bilirubin), pruritus (from bile salts), or xanthomas (from lipid deposits in skin). Absence of bile salts to dissolve fat-soluble nutrients can lead to deficiencies of fat-soluble vitamins A, D, E, and K. This may result in osteoporosis, due to lack of vitamin D, and PT/INR elevation, due to lack of vitamin K.

Cholestatic syndromes may be subclassified as either disorders of hepatocytes and microscopic bile ducts (*intrahepatic cholestasis*) or anatomic obstructions to macroscopic bile ducts (*extrahepatic cholestasis*).[20] The approach to a patient with cholestasis generally begins with a radiographic study, often a right upper-quadrant ultrasound, to look for dilation of large bile ducts within or outside the liver. Dilation of such large bile ducts indicates extrahepatic cholestasis; otherwise extrahepatic cholestasis is largely excluded, and the next step is to investigate for various causes of intrahepatic cholestasis.

Intrahepatic Cholestasis

Intrahepatic cholestasis includes a variety of processes that interfere with hepatocyte secretion of bile, as well as diseases of the microscopic and macroscopic bile ducts within the liver. Etiologies involving impaired hepatocyte secretion of bile overlap to some extent with hepatocellular diseases as noted above; such processes include viral hepatitis (especially type A), alcoholic hepatitis, and even cirrhosis. Processes that cause a more pure cholestatic pattern include a variety of drugs (**Table 15-2**), pregnancy, severe infection (cholestasis of sepsis), and certain nonhepatic neoplasms, especially renal cell carcinoma. Infiltrative processes of the liver will produce a primarily cholestatic pattern, and these include granulomatous diseases and amyloidosis. Primary biliary cholangitis (PBC) causes inflammatory scarring of the microscopic bile ducts, whereas sclerosing cholangitis is a similar process that may affect microscopic or macroscopic bile ducts within the liver. Masses within the liver, including tumors or abscesses, may block the flow of bile as well.

Extrahepatic Cholestasis

Extrahepatic cholestasis involves obstruction of the large bile ducts outside of the liver. The most common cause is stones in the common bile duct; other causes include obstruction by strictures (after surgery), tumors (of the pancreas, ampulla

TABLE 15-2. Classification of Liver Disease[a]

HEPATOCELLULAR

Viral hepatitis
Autoimmune hepatitis
Impaired blood flow
- Hypotension (shock liver)
- Congestive heart failure

Metabolic diseases
- Hemochromatosis
- Wilson disease
- Alcoholic hepatitis
- NAFLD

Drugs include

Acetaminophen
ACE Inhibitors
Allopurinol
Amiodarone
Antiepileptic agents
- Carbamazepine
- Phenytoin
- Valproic acid

Antimicrobial agents
- Amoxicillin– clavulanate
- Azole antifungals
- Dapsone
- Fluoroquinolones
- INH
- Nitrofurantoin
- Protease inhibitors
- Sulfonamides

Azathioprine
Cisplatin
Glyburide
Heparin
Labetalol
Methotrexate
Methyldopa
Niacin
NSAIDs
Phenothiazines
Trazodone
Statin medications
Herbal medications
Nutritional supplements
Illicit drugs
Toxins

CHOLESTATIC

Intrahepatic
Cholestasis of sepsis
Extra-hepatic neoplasms (i.e., renal cell)
Cholestasis of pregnancy
Infiltrative liver diseases
- Granulomatous (sarcoid, TB)
- Lymphoma
- Metastatic carcinoma

Inflammatory diseases of bile ducts
- PBC
- PSC

Drugs include

Allopurinol
Antibiotics
- Erythromycin
- β-lactams
- Rifampin

Cardiovascular
- Amiodarone
- Captopril
- Diltiazem
- Quinidine

Carbamazepine
Hormonal agents
- Estrogens
- Methyltestosterone
- Anabolic steroids

Niacin
NSAIDs
Penicillamine
Phenothiazines
Sulfa drugs
TPN (hyperalimentation)

Extrahepatic
Biliary stricture
Gallstone-obstructing bile duct
Tumors
- Pancreatic cancer
- Cholangiocarcinoma of bile duct

PSC
AIDS cholangiopathy

ACE = angiotensin-converting enzyme; AIDS = acquired immune deficiency syndrome; INH = isoniazid; NAFLD = nonalcoholic fatty liver disease; NSAIDs = nonsteroidal anti-inflammatory drugs; PBC = primary biliary cholangitis; PSC = primary sclerosing cholangitis; TB = tuberculosis; TPN = total parenteral nutrition.

[a]Please note that listings of drugs contain more commonly used agents and are not exhaustive. For any particular patient, potentially causative drugs should be specifically researched in appropriate databases to determine any hepatotoxic effects, perhaps the best of these being the National Library of Medicine data base, LiverTox, which can be accessed: Livertox.nih.gov/.

of Vater, duodenum, or bile ducts), chronic pancreatitis with scarring of the ducts as they pass through the pancreas, and parasitic infections of the ducts. Another cause is primary sclerosing cholangitis (PSC), a disease causing diffuse inflammation of the bile ducts, often both intrahepatic and extrahepatic. Of note is that PSC is associated with inflammatory bowel disease, especially involving the colon. Some patients with HIV can develop a picture similar to sclerosing cholangitis, referred to as *AIDS cholangiopathy.* Although previously referred to as *surgical cholestasis*, extrahepatic cholestasis can now often be treated or at least palliated using endoscopic means (e.g., dilation of strictures with or without stent placement). Another entity is IgG4-related sclerosing cholangitis. This is an autoimmune disease, a variant of autoimmune hepatitis, often with elevated autoimmune markers (antinuclear antibody, abnormal serum protein electrophoresis). It can present with a picture of sclerosing cholangitis or even cholangiocarcinoma, but the elevated autoimmune markers, especially elevated levels of IgG4, help make this distinction. Tissue biopsy will reveal IgG4, plasma cell infiltrates, and interstitial fibrosis. These patients will characteristically respond to glucocorticoids.

Tests Associated with Excretory Liver Function and Cholestasis

Laboratory tests do not distinguish between intrahepatic and extrahepatic cholestasis. This distinction is usually made radiographically. In most instances of extrahepatic cholestasis, a damming effect causes dilation of bile ducts above the obstruction, which can be visualized via a computed tomography (CT) scan, a magnetic resonance imaging (MRI) scan, or ultrasound. Laboratory abnormalities primarily associated with cholestasis include elevation of ALP, 5'-nucleotidase, γ-glutamyl transpeptidase (GGT), and bilirubin.

Alkaline Phosphatase

Normal range: 33–96 units/L (0.55–1.6 μkat/L)

Alkaline phosphatase (ALP) refers to a group of isoenzymes whose exact function remains unknown. These enzymes are found in many body tissues including the liver, bone, small intestine, kidneys, placenta, and leukocytes. In the liver they are found primarily in the bile canalicular membranes of the liver cells. In adults, most serum ALP comes from the liver and bone (~80%), with the remainder mostly contributed by the small intestine.

Normal ALP concentrations vary primarily with age. In children and adolescents, elevated ALP concentrations result from bone growth, which may be associated with elevations as high as three times the adult normal range. Similarly, the increase during late pregnancy is due to placental ALP.[21,22] In the third trimester, concentrations often double and may remain elevated for three weeks postpartum.

The mechanism of hepatic ALP release into the circulation in patients with cholestatic disease remains unclear. Bile accumulation appears to increase hepatocyte synthesis of ALP, which eventually leaks into the bloodstream.[8,24] The ALP concentrations persist until the obstruction is removed and then normalize within two to four weeks.

Clinically, ALP elevation is associated with cholestatic disorders and, as mentioned previously, does not help to distinguish between intrahepatic and extrahepatic disorders. ALP concentrations more than four times normal suggest a cholestatic disorder, and 75% of patients with primarily cholestatic disorders have ALP concentrations in this range (**Table 15-3**). Concentrations of three times normal or less are nonspecific and can occur in all types of liver disease. Mild elevations, usually <1½ times normal, can be seen in normal patients and are less significant.

When faced with an elevated ALP concentration, a clinician must determine whether it is derived from the liver. One approach is to fractionate the ALP isoenzymes using electrophoresis, but this method is expensive and often unavailable. Thus, the approach usually taken is to measure other indicators of cholestatic disease, 5'-nucleotidase, or GGT. If ALP is elevated, an elevated 5'-nucleotidase or GGT indicates that at least part of the elevated ALP is of hepatic origin. Alternatively, a normal 5'-nucleotidase or GGT suggests a nonhepatic cause (Table 15-3).

Nonhepatic causes of elevated ALP include bone disorders (e.g., healing fractures, osteomalacia, Paget disease, rickets, tumors, osteoporosis, hypervitaminosis D, or vitamin D deficiency as caused by celiac sprue), hyperthyroidism, hyperparathyroidism, sepsis, diabetes mellitus, renal failure, and neoplasms (which may synthesize ALP ectopically, outside tissues that normally contain ALP) (**Table 15-4**). Some families have inherited elevated concentrations (two to four times normal), usually as an autosomal dominant trait.[25] Markedly elevated concentrations (greater than four times normal) are generally seen only in cholestasis, Paget disease, or infiltrative

TABLE 15-3. Initial Evaluation of Elevated ALP Concentrations in Context of Other Test Results

ALP	GGT, 5' NUCLEOTIDASE	AMINOTRANSFERASES (ALT AND AST)	DIFFERENTIAL DIAGNOSIS
Mildly elevated	Within normal limits	Within normal limits	Pregnancy; nonhepatic causes (Table 15-4)
Moderately elevated[a]	Markedly elevated	Within normal limits or minimally elevated	Cholestatic syndromes
Mildly elevated[b]	Mildly elevated	Markedly elevated	Hepatocellular disease

ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; GGT = gamma-glutamyl transpeptidase.

[a] Usually greater than 4 times normal limits.

[b] Usually less than 4 times normal limits.

TABLE 15-4. Some Nonhepatic Illnesses Associated with Elevated ALP

BONE DISORDERS	OTHER DISORDERS AND DRUGS
Healing fractures	Acromegaly
Osteomalacia	Anticonvulsant drugs (e.g., phenytoin and phenobarbital)
Paget disease	Hyperthyroidism
Rickets	Lithium (bone isoenzymes)
Tumors	Neoplasia
	Oral contraceptives
	Renal failure
	Small bowel obstruction
	Pregnancy
	Sepsis

diseases of the liver. Because of an increase in intestinal ALP, serum ALP concentrations can be falsely elevated in patients of blood type O or B whose blood is drawn two to four hours after a fatty meal.[26] Alkaline phosphatase concentrations can be lowered by a number of conditions including hypothyroidism, hypophosphatemia, pernicious anemia, and zinc or magnesium deficiency.[8] Also, ALP may be confounded by a variety of drugs.

5'-Nucleotidase

Normal range: 0–11 units/L (0–0.18 μkat/L)

Although *5'-nucleotidase* is found in many tissues (including liver, brain, heart, and blood vessels), serum 5'-nucleotidase is elevated most often in patients with hepatic diseases.[19] It has a response profile parallel to ALP and similar utility in differentiating between hepatocellular and cholestatic liver disease. Because it is only elevated in the face of liver disease, the presence of an elevated ALP together with a normal 5'-nucleotidase suggest that the ALP is elevated secondary to nonhepatic causes.

Gamma-Glutamyl Transpeptidase

Normal range: 9–58 units/L (0.15–0.97 μkat/L)

Gamma-glutamyl transpeptidase (GGT, also GGTP*),* a biliary excretory enzyme, can also help determine whether an elevated ALP is of hepatic etiology. Similar to 5'-nucleotidase, it is not elevated in bone disorders, adolescence, or pregnancy. It is rarely elevated in conditions other than liver disease.

Generally, GGT parallels ALP and 5'-nucleotidase levels in liver disease. Additionally, GGT concentrations are usually elevated in patients who abuse alcohol or have alcoholic liver disease. Therefore, this test is potentially useful in differential diagnosis with a GGT/ALP ratio >2.5 being highly indicative of alcohol abuse.[16,27] With abstinence, GGT concentrations often decrease by 50% within two weeks.

Although it is often regarded as the most sensitive test for cholestatic disorders, GGT is unlike 5'-nucleotidase in that GGT lacks specificity. In one study of nonselected patients, only 32% of GGT elevations were of hepatic origin.[18] GGT is found in the liver, kidneys, pancreas, spleen, heart, brain, and seminal vesicles. Elevations may occur in pancreatic diseases, myocardial infarction, severe chronic obstructive pulmonary diseases, some renal diseases, systemic lupus erythematosus, hyperthyroidism, certain cancers, rheumatoid arthritis, and diabetes mellitus. GGT may be confounded in patients on a variety of medications, some of which overlap with the medications that confound ALP test results. Thus, elevated GGT (even with concomitant elevated ALP) does not necessarily imply liver injury when 5'-nucleotidase is normal, but rather both elevations in GGT and ALP may be due to a common confounding medication (e.g., phenytoin, barbiturates) or medical condition (e.g., myocardial infarction).

Bilirubin

Total bilirubin: 0.3–1.3 mg/dL (5.1–22.2 μmol/L)
Indirect (unconjugated, insoluble) bilirubin: 0.2–0.9 mg/dL (3.4–15.4 μmol/L)
Direct (conjugated, water soluble) bilirubin: 0.1–0.4 mg/dL (1.7–6.8 μmol/L)

Understanding the various laboratory studies of *bilirubin* requires knowledge of the biochemical pathways for bilirubin production and excretion (**Figure 15-3**). Bilirubin is a breakdown product of heme pigments, which are large, insoluble organic compounds. Most of the body's heme pigments are located in erythrocytes (red blood cells) where they are a component of hemoglobin. Breakdown of erythrocytes releases hemoglobin into the circulation (which is converted to bilirubin, predominantly in the spleen) where it is initially a large lipophilic molecule bound to albumin.

The liver plays a central role in excretion of bilirubin, similar to its role in the metabolism and excretion of a wide variety of lipophilic substances. Prior to excretion, bilirubin must be converted into a form that is water-soluble. The liver achieves this by covalently linking it to a water-soluble sugar molecule (glucuronic acid) using an enzyme glucuronyl transferase. The conjugate of bilirubin linked to glucuronic acid is water soluble, so it may then be excreted into the bile and eventually eliminated in the feces. Incidentally, bilirubin and some of its breakdown products are responsible for coloring feces brown (such that with complete obstruction of the bile ducts or cessation of bile synthesis by the liver, stool will take on a pale color).

Indirect Versus Direct Bilirubin

The total amount of bilirubin in the serum can be divided into *direct* and *indirect* fractions. Bilirubin conjugated to glucuronic acid (water-soluble bilirubin) reacts quickly in the van der Bergh reaction and is thus called *direct-reacting* or *direct bilirubin.* Alternatively, unconjugated bilirubin, because it is water insoluble, requires the presence of dissolving agents to be detected by this assay and is thus called *indirect-reacting* or *indirect bilirubin.* Although this nomenclature system is slightly awkward, it is the standard terminology used in clinical practice today. Only the water-soluble direct bilirubin can be excreted in the urine, and therefore urine dipsticks only will measure this fraction. In fact, urine dipsticks may be more sensitive than most serum tests for detecting a slight elevation of direct bilirubin.

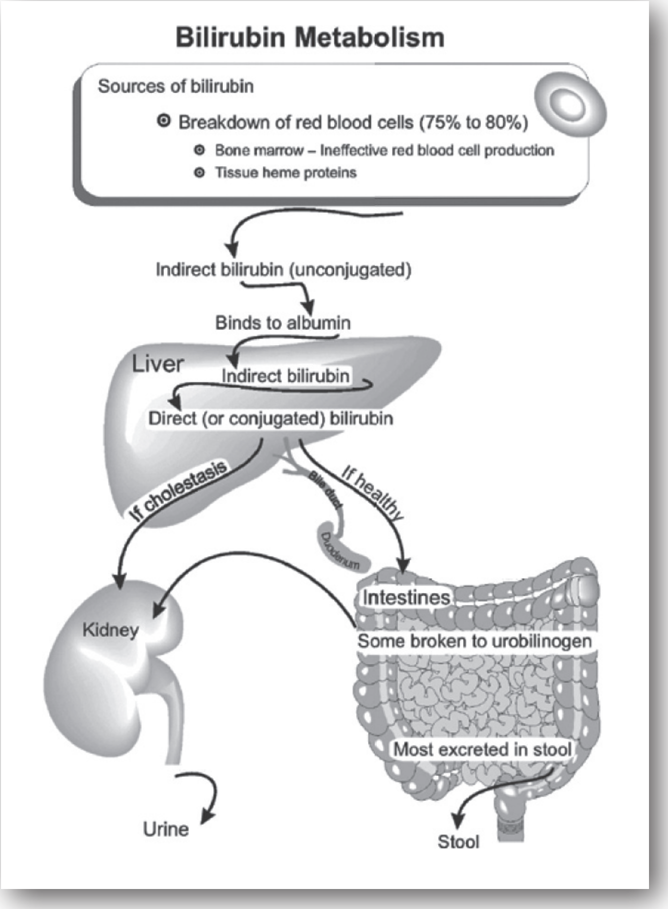


FIGURE 15-3. Overview of bilirubin production and metabolism. Most bilirubin is produced by the breakdown of heme pigments in erythrocytes (red blood cells) and to a lesser extent other tissues. The indirect bilirubin is carried in the circulation to the liver where it is conjugated and becomes direct or conjugated bilirubin. In healthy individuals, conjugated bilirubin is largely excreted via the biliary system into the gut. In diseased individuals, it will "back up" into the circulation, causing elevated levels of direct/conjugated bilirubin and ultimately jaundice. (With thanks to Esta Farkas.)

Elevated bilirubin causes abnormal yellow coloration of the skin and sclera of the eyes (collectively, these symptoms are referred to as jaundice or icterus). Excess carotenes (as due to large amounts of carrot consumption) may cause a similar effect on the skin but spare the eyes. Icterus usually becomes visible when total bilirubin concentrations exceed 2–4 mg/dL. In infants, extremely elevated concentrations of bilirubin (for example, >20 mg/dL) may have neurotoxic effects on the developing brain, but in adults a direct toxic effect of bilirubin is quite rare.[28]

The first step in evaluating an elevated serum bilirubin is to determine if only the indirect fraction is elevated, or if there is involvement of the direct fraction. Given the sequential location of these two molecules within the pathway of bilirubin metabolism, elevated levels of the molecules may have markedly different significance (**Table 15-5**).

Indirect Hyperbilirubinemia (unconjugated, insoluble)

Indirect bilirubin is mostly produced by the breakdown of erythrocytes and is removed from the circulation by conversion to direct bilirubin by glucuronyl transferase in the liver. Therefore, elevated levels may result from increased breakdown of red blood cells (hemolysis) or reduced hepatic conversion to direct bilirubin.[18] Patients with primarily *unconjugated hyperbilirubinemia* (>70% indirect) generally do not have serious liver disease. The most common causes of elevated indirect bilirubin are hemolysis, Gilbert syndrome, Crigler-Najjar syndrome, or various drugs, including probenecid and rifampin.[29] In infants this can be physiologic (neonatal jaundice), although very high levels may require medical intervention.

Hemolysis refers to increased destruction of erythrocytes, which increases the production of indirect bilirubin and may overwhelm the liver's ability for conjugation and excretion. However, the liver's processing mechanisms are intact so that serum bilirubin generally does not rise dramatically (rarely >5 mg/dL). Hemolysis may result from a wide variety of hematologic processes including sickle cell anemia, spherocytosis, hematomas, mismatched blood transfusions,

TABLE 15-5. Evaluation of Elevated Bilirubin Concentrations in Context of Other Test Results

TOTAL BILIRUBIN	DIRECT BILIRUBIN	INDIRECT BILIRUBIN	ALT, AST, GGT	DIFFERENTIAL DIAGNOSIS
Moderately elevated	Within normal limits or low	Moderately elevated	Within normal limits	Hemolysis,[a] Gilbert syndrome,[a] Crigler-Najjar syndrome,[b] Neonatal jaundice
Moderately elevated	Moderately elevated	Within normal limits	Within normal limits	Congenital syndromes[c]: Dubin-Johnson,[d] Rotor
Mildly elevated	Mildly elevated	Moderately elevated	Moderately elevated	Hepatobiliary disease

ALT = alanine aminotransferase; AST = aspartate aminotransferase; GGT = gamma-glutamyl transpeptidase.

[a] Usually indirect bilirubin is <4 mg/dL but may increase to 18 mg/dL.

[b] Usually indirect bilirubin is >12 mg/dL and may go as high as 45 mg/dL.

[c] These syndromes are distinguished in the laboratory by liver biopsy.

[d] Usually direct bilirubin is 3–10 mg/dL.

or intravascular fragmentation of blood cells. Evaluation will include various hematologic tests as described in further detail in chapter 16.

Gilbert syndrome is an inherited, benign trait present in 3–5% of the population. It is due to reduced production of hepatic glucuronyl transferase enzymes, resulting in intermittent elevation of indirect bilirubin and mild jaundice (increased with fasting, stress, or illness). The primary significance is that it may cause elevation of bilirubin when there is in fact no significant hepatic or hematologic disease. Bilirubin elevation is generally mild, with values <5 mg/dL.[2]

Direct Hyperbilirubinemia (conjugated, soluble)

Conjugated hyperbilirubinemia is defined as bilirubinemia with >50% in the direct fraction (although absolute levels of unconjugated bilirubin may also be elevated).[17] In the normal course of bilirubin metabolism, direct bilirubin is synthesized in hepatocytes by conjugating indirect bilirubin, and secreted into bile. Therefore, elevated direct bilirubin implies hepatic or biliary tract disease that interferes with secretion of bilirubin from the hepatocytes or clearance of bile from the liver.

Direct hyperbilirubinemia is generally classified as a positive cholestatic liver test, although as discussed earlier, it may be elevated to some extent in hepatocellular processes as well. In cholestatic disease, elevated bilirubin is primarily conjugated, whereas in hepatocellular processes significant increases in both conjugated and unconjugated bilirubin may result. The most reliable method of determining the cause of hyperbilirubinemia considers the magnitude and pattern of abnormalities in the entire liver function panel. It should be noted that direct bilirubin is generally readily cleared by the kidney, such that its levels rarely rise very high even in severe cholestatic disease if the patient has normal renal function. Very rarely, congenital disorders (e.g., Dubin-Johnson and Rotor syndromes) may cause elevations of primarily conjugated bilirubin.

It should be noted that a gray area exists between indirect and direct hyperbilirubinemia. Most authors agree that >50% direct bilirubin indicates direct hyperbilirubinemia whereas <30% direct fraction indicates indirect hyperbilirubinemia.[2] For cases where the fraction falls between 30–50%, other liver tests and hematologic tests may be required to determine the etiology. Patients with elevated direct bilirubin levels may have some binding of bilirubin to albumin, referred to as *delta bilirubin*. This explains delayed resolution of jaundice during recovery from acute hepatobiliary diseases, while the “free” bilirubin is rapidly metabolized, the bilirubin linked to albumin is metabolized at a much slower rate. Delta bilirubin has a half-life of 14–21 days, which is similar to albumin.[30]

HEPATOCELLULAR INJURY

As discussed earlier, the liver is a large organ with diverse biochemical roles, which require its cells to be in close communication with the bloodstream. These properties place the hepatocytes at risk of injury due to a variety of processes. Toxin and drug metabolism produce cascades of metabolic byproducts, some of which may damage hepatocytes. Likewise, the liver plays a central role in the body’s biochemical homeostasis, so metabolic disorders tend to involve the liver. Finally, the close relationship of hepatocytes to the blood supply places them at risk for a variety of infectious agents.

Hepatitis is a term that technically refers to a histologic pattern of inflammation of hepatocytes. It may also be used to refer to a clinical syndrome due to diffuse liver inflammation. The laboratory reflection of hepatitis is a hepatocellular injury pattern, which is marked primarily by elevated aminotransferases.

There are multiple causes of hepatitis. One common type is viral hepatitis, which is classified A, B, C, D (delta hepatitis), E, or G based on the causative virus. These viruses, and the tests for them, are discussed in detail in the Viral Hepatitis section. Less common viral hepatitis may be caused by the Epstein-Barr virus, herpes virus, or cytomegalovirus.

Hepatitis may also be caused by various medications, and drug-induced hepatitis can be either acute or chronic.[28] Some drugs commonly implicated in cellular hepatotoxicity are listed in Table 15-2. In addition, elevation of aminotransferases has been reported in patients receiving heparin.[31] ALT is elevated in up to 60% of these patients, with a mean maximal value of 3.6 times the baseline. A vast number of drugs can cause hepatic injury, especially drugs that are extensively metabolized by the liver. Although numerous drugs may result in aminotransferase elevations, such elevations are usually minor, transient, not associated with symptoms, and of no clinical consequence.[32]

Perhaps the most common cause of abnormal aminotransferases in ambulatory patients is fatty liver. Estimates are 30–40% of adults in the United States have fatty liver, which can vary from hepatic steatosis (fat in the liver) to nonalcoholic steatohepatitis (NASH), where the extra fat in the liver is associated with inflammation. It is potentially serious as up to one quarter of these patients can progress to cirrhosis. Fatty liver and NASH are mostly related to increased body mass index, but they can also be associated with rapid weight loss or drugs such as tamoxifen, amiodarone, diltiazem, nifedipine, corticosteroids, and petrochemicals. Fatty liver/NASH can be seen in hepatitis C and patients on TPN, and it is also associated with hypothyroidism and short bowel syndrome. It is important to note that although mild hepatic inflammation is often of minimal significance, it may signal the presence of a chronic and serious disease process. Some other causes of hepatic inflammation and injury are listed in Table 15-2.

It is often difficult to determine the exact etiology of hepatic inflammation or hepatitis. A careful history—especially for exposure to drugs, alcohol, or toxins—and detailed physical examination are crucial. Additional laboratory studies are usually necessary to distinguish one form of hepatitis from another (**Figure 15-4**). Radiological testing or liver biopsy may be indicated, not only to determine the etiology of the liver disease, but also to help determine the indications for (and results of) therapy and prognosis.

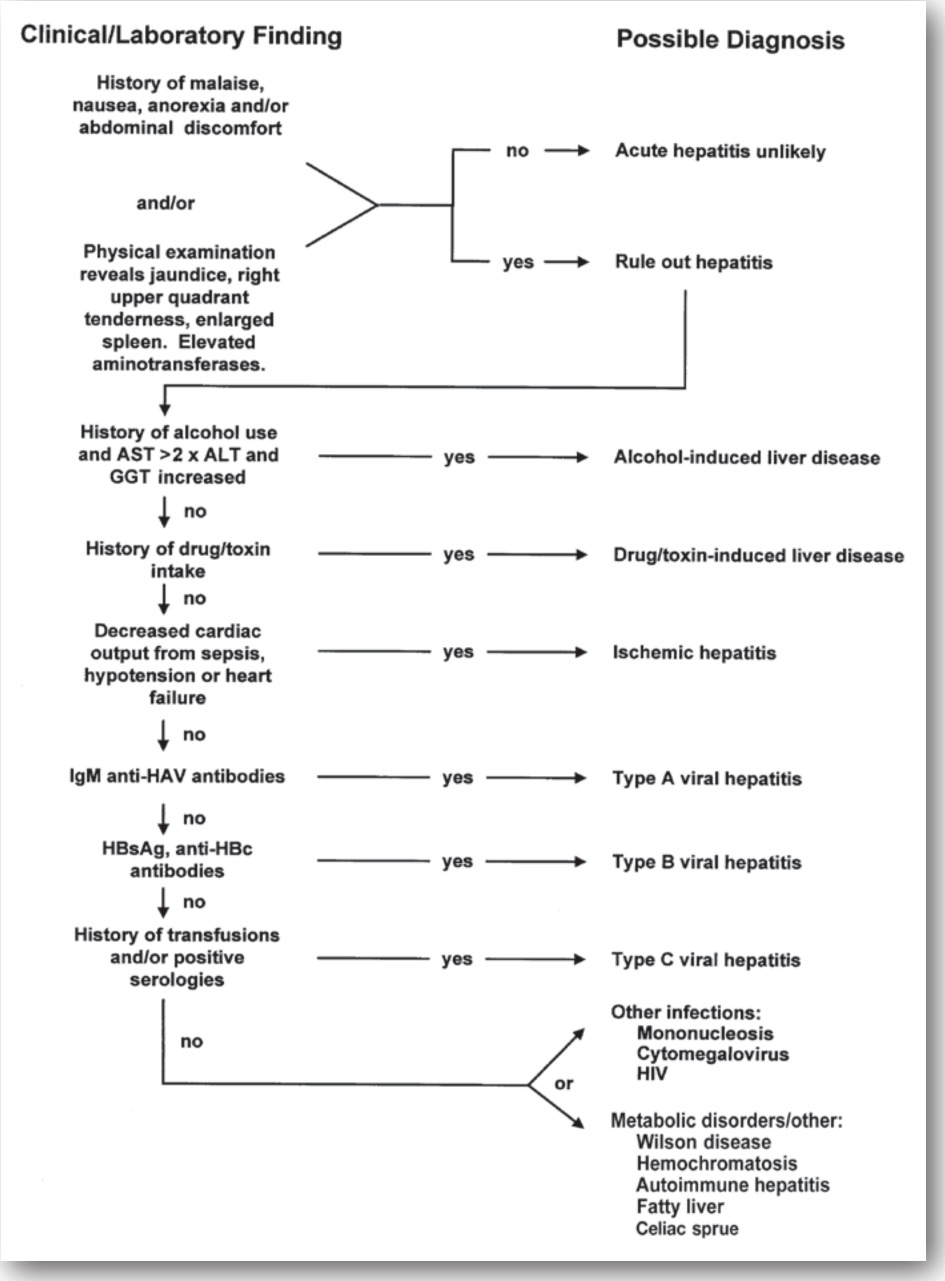

Clinical/Laboratory Finding
Possible Diagnosis
History of malaise, nausea, anorexia and/or abdominal discomfort
and/or
Physical examination reveals jaundice, right upper quadrant tenderness, enlarged spleen. Elevated aminotransferases.
no
Acute hepatitis unlikely
yes
Rule out hepatitis
History of alcohol use and AST >2 x ALT and GGT increased
yes
Alcohol-induced liver disease
no
History of drug/toxin intake
yes
Drug/toxin-induced liver disease
no
Decreased cardiac output from sepsis, hypotension or heart failure
yes
Ischemic hepatitis
no
IgM anti-HAV antibodies
yes
Type A viral hepatitis
no
HBsAg, anti-HBc antibodies
yes
Type B viral hepatitis
no
History of transfusions and/or positive serologies
yes
Type C viral hepatitis
no
or
Other infections:
Mononucleosis
Cytomegalovirus
HIV
Metabolic disorders/other:
Wilson disease
Hemochromatosis
Autoimmune hepatitis
Fatty liver
Celiac sprue

FIGURE 15-4. Algorithm for differential diagnosis of suspected hepatitis.

Aminotransferases: AST and ALT

AST: 12–38 units/L (0.2–0.64 μkat/L); ALT: 7–41 units/L (0.12–0.68 μkat/L) (normal values for either test vary from laboratory to laboratory but tend to be in the range of <30 units/L for men and <20 units/L for women)

The *aminotransferases* (also known as *transaminases*) are used to assess hepatocellular injury and include AST (formerly serum glutamic-oxaloacetic transaminase or SGOT) and ALT (formerly serum glutamic-pyruvic transaminase or SGPT. These enzymes are primarily located inside hepatocytes, where they assist with various metabolic pathways. They are released into the serum in greater quantities when there is hepatocyte damage, are very sensitive, and may be elevated even with minor levels of hepatocyte damage.[33] However, this renders them relatively nonspecific, and slightly elevated levels may not be clinically significant (particularly in an ill, hospitalized patient who is on many medications and has a variety of active medical problems).

Aminotransferases will often be slightly increased in cholestatic liver diseases, but in this situation they will generally be overshadowed by a greater elevation of cholestatic liver tests (i.e., ALP and total bilirubin to produce a predominantly cholestatic pattern of liver tests). If both aminotransferases and cholestatic tests are elevated in a similar pattern, it suggests a severe hepatocellular process, which interferes with bile secretion at the level of the hepatocytes. Finally, it should be noted that aminotransferases may rise into the thousands within 24–48 hours following common bile duct obstruction, after which they decline rapidly. This is one instance in which a cholestatic process may transiently cause a hepatocellular injury LFT profile.

Both AST and ALT have half-lives of 17 and 47 hours, respectively, so they reflect *active* hepatocyte damage, and not, for example, damage to hepatocytes that occurred weeks, months, or years previously. This may lead to some counterintuitive relationships between aminotransferase levels and the overall state of the liver. For example, a drop in aminotransferase levels in the setting of acute massive (*fulminant*) hepatitis may reflect a depletion of viable hepatocytes with poor prognosis.[18] Extremely high concentrations (>1000 units/L) are usually associated with acute viral hepatitis, severe drug or toxic reactions, or ischemic hepatitis (inadequate blood flow to the liver). Lesser elevations are caused by a vast number of hepatic insults and are less specific.[34]

The ratio of AST to ALT may be of value in diagnosing alcoholic hepatitis, where the AST is generally at least twice the ALT, and the AST is rarely >300 units/L. In alcoholic liver disease, this is due, in part to a deficiency of pyridoxal 5'-phosphate, which favors production of ALT over AST.[35] Alcoholic liver disease is also suggested by an elevation in GGT as previously reviewed.

AST is not solely located in hepatocytes but rather is also found in cardiac muscle, skeletal muscle, kidneys, brain, lungs intestines, and erythrocytes. Consequently, AST may be elevated due to a variety of situations including musculoskeletal diseases (e.g., muscular dystrophy, dermatomyositis, heavy exercise, trichinosis, gangrene, and muscle damage secondary to hypothyroidism), myocardial infarction, renal infarction or failure, brain trauma or cerebral infarction, hemolysis, pulmonary embolism, necrotic tumors, burns, and celiac sprue.[6,7,18] ALT is more localized to the liver than AST, so it is more specific to liver injury. Elevation of AST without elevation of the ALT or other liver test abnormality suggests cardiac or muscle disease.[17] A muscular origin of aminotransferases may also be indicated by increases in aminotransferases above 300 IU/L with concomitant increases in serum creatine kinase activity.[25,36]

Measurement of AST may be affected by a bewildering variety of medications. Almost any prescription drug (as well as various herbal compounds and illegal drugs) can cause an elevation of aminotransferases, and the significance of these elevations is often unclear.[26] Furthermore, the in vitro assay may be confounded by a variety of factors including uremia, hyperlipidemia, and hemolysis.[37] False elevations in the in vitro test may also be seen in patients on acetaminophen, levodopa, methyldopa, tolbutamide, para-aminosalicylic acid, or erythromycin.[8,38,39]

Other factors may interfere with the test's accuracy. Levels may be elevated to two to three times normal by vigorous exercise in males and decreased to about half following dialysis.[7] Complexing of AST with immunoglobulin (known as *macro-AST*) may occasionally produce a clinically irrelevant elevation of AST.[40] Testing for macro-AST is not a clinical laboratory test used in practice. Given the array of factors that can cause an abnormal result, unexplained false positives often occur. In healthy individuals, an isolated elevated ALT returns to normal in repeat studies one half to one third of the time.[41] For this reason, prior to an evaluation of mildly elevated aminotransferases in low-risk healthy patients, a practitioner should check for an elevation of more than one test (i.e., both AST and ALT) or repeated elevations of a single test.

TESTS ASSOCIATED WITH DETOXIFICATION

Hepatic Encephalopathy

Hepatic encephalopathy refers to a diffuse metabolic dysfunction of the brain that may occur in acute or chronic liver failure. Clinically it ranges from subtle changes in personality to coma and death. The etiology of hepatic encephalopathy remains controversial and has undergone significant revision recently. Many theories ascribe a major role to ammonia. The majority of serum ammonia enters the blood from the intestines, where it is formed by bacterial catabolism of protein within the gut lumen as well as conversion of serum glutamine into ammonia by enterocytes of the small intestine.[42] Normally, the liver removes >90% of this ammonia via first-pass metabolism.[43] In liver failure, ammonia, along with possibly other toxic substances may avoid this first-pass metabolism and gain immediate access to the brain where it has a variety of toxic effects.[44] Although serum ammonia is currently the "standard" laboratory test for assessing hepatic encephalopathy, other tests are being developed. Elevated serum levels of 3-nitro-tyrosine may be a marker for minimal hepatic encephalopathy (cirrhotic patients with mild cognitive impairment). In a pilot study using a cutoff of 14 micromole/mol, 3-nitro-tyrosine levels had a 93% sensitivity and an 89% specificity in identifying these patients.[45]

Ammonia

Normal range[3]: 19–60 mcg/dL (13.6–42.8 µmol/L)

Ammonia levels do not correlate well with hepatic encephalopathy in the setting of chronic liver failure (i.e., patients with cirrhosis). This is likely because hepatic encephalopathy also involves an increase in the permeability of the blood–brain barrier to ammonia.[6,46] There is a large overlap between ammonia levels in patients with and without hepatic encephalopathy among patients with chronic liver disease making it a poor test in this situation.[47] A very high ammonia level (i.e., >250 mcg/dL) is suggestive of hepatic encephalopathy, but most cirrhotic patients suspected of having encephalopathy will have normal or slightly elevated ammonia levels, which adds little diagnostic information. In this situation, hepatic encephalopathy is a clinical diagnosis based on history, physical exam, and exclusion of other possibilities. Psychometric testing is becoming increasingly available to assist in confirming this diagnosis.

Some recent studies have suggested that ammonia may have more significance in the setting of acute liver failure (e.g., due to overwhelming infection of the liver by viral hepatitis). In these patients, the degree of ammonia elevation correlates with the severity of hepatic encephalopathy and the likelihood of death, and it may be a useful marker for predicting which patients require emergent liver transplant.[48,49]

Ammonia concentration may also be elevated in patients with Reye syndrome, inborn disorders of the urea cycle, various medications (most notably valproic acid), impaired renal function, ureterosigmoidostomy, or urinary tract infections with bacteria that convert urea to ammonia. In cirrhotic patients or patients with mild liver disease, elevated ammonia and hepatic encephalopathy may be precipitated by such factors as increased dietary protein, GI bleeding, constipation, and *H. pylori* infection.[7]

VIRAL HEPATITIS

The onset of acute *viral hepatitis* may be quite dramatic and present as an overwhelming infection, or it may pass unnoticed by the patient. In the usual prodromal period, the patient often has a nonspecific flu-like illness that may include nausea, vomiting, fatigue, or malaise. This period may be followed by clinical hepatitis with jaundice. During this time, the most abnormal laboratory studies are usually the aminotransferases, which can be in the thousands. Bilirubin may be quite elevated, while ALP only mildly so.

The major types of viral hepatitis are reviewed here, but they are often clinically indistinguishable. Thus, serologic studies of antibodies, molecular assays to detect viral genetic material, and knowledge of the epidemiology and risk factors for these different viruses (**Table 15-6**) are central to diagnosis.

Type A Hepatitis

Hepatitis A virus (HAV) is spread primarily by the fecal–oral route by contaminated food or water or by person-to-person contact. It has an incubation period of three to five weeks with a several-day prodrome (preicteric phase) before the onset of jaundice and malaise, or the icteric phase. The icteric phase generally lasts one to three weeks, although prolonged courses do occur. Hepatitis A is responsible for about 50% of acute hepatitis in the United States (more than all other hepatotropic viruses combined), generally due to person-to-person contact within community-wide outbreaks.[50,51] Interestingly the incidence of new cases has diminished with the widespread use of the HAV vaccine (see below).

Unlike types B, C, and D hepatitis virus, HAV does not cause chronic disease, and recovery usually occurs within one month. Many patients who get type A hepatitis never become clinically ill. Perhaps 10% of all patients become symptomatic, and only 10% of those patients become jaundiced.[4] The majority of patients have a full recovery, but there is a substantial mortality risk in elderly patients and very young patients, and patients with chronic hepatitis B or C, and those with chronic liver disease of other etiologies.[52-54]

TABLE 15-6. Groups at Higher Risk of Infection by Various Hepatitis Viruses

- Hepatitis A virus
  - Contacts with infected persons
  - Daycare workers and attendees
  - Institutionalized persons
  - Travelers to countries with high rate of hepatitis A infections
  - Military personnel
  - Men who have sex with men
  - IV drug users
- Hepatitis B virus
  - Contacts of infected persons
  - Unvaccinated healthcare professionals and morticians
  - Hemodialysis patients
  - Male homosexuals
  - IV drug users
  - Multipartner heterosexuals
  - Tattooed/body-pierced persons
  - Newborns of HBsAg-carrier mothers
- Hepatitis C virus
  - Dialysis patients
  - Healthcare professionals
  - IV drug users (primary cause) (even once)
  - Snorting cocaine
  - Recipients of clotting factors before 1987
  - Recipients of transfusion or organ transplant before July 1992
  - Tattooed/body-pierced persons
  - Children born to HCV-positive mothers
- Hepatitis D virus
  - Only individuals with chronic HBV infection
- Hepatitis E virus
  - Travelers to Latin America, Egypt, India, and Pakistan
  - Contacts with infected persons

HBsAg = surface antigen of the hepatitis B virus; HBV = hepatitis B virus; HCV = hepatitis C virus; IV = intravenous.

A vaccine for HAV is now available. It is recommended for those from or traveling to endemic regions (i.e., Central and South America), men who have sex with men, users of street drugs, those with occupational exposure, patients requiring clotting factor concentrates, and patients with chronic liver diseases. This vaccine is increasingly being recommended as a universal vaccine for pediatric patients. Although this vaccine is generally preferred for postexposure prophylaxis, use of immunoglobulin should be considered in the very young (under 12 months) or patients who cannot receive the vaccine.

Presently, the only two tests available to measure antibodies to HAV are immunoglobulin M (IgM) or total (all isotypes of) antibody. Detection of IgM is the more clinically relevant test as it reveals acute or recent infection. These antibodies are present at the onset of jaundice and decline within 12 (but usually 6)

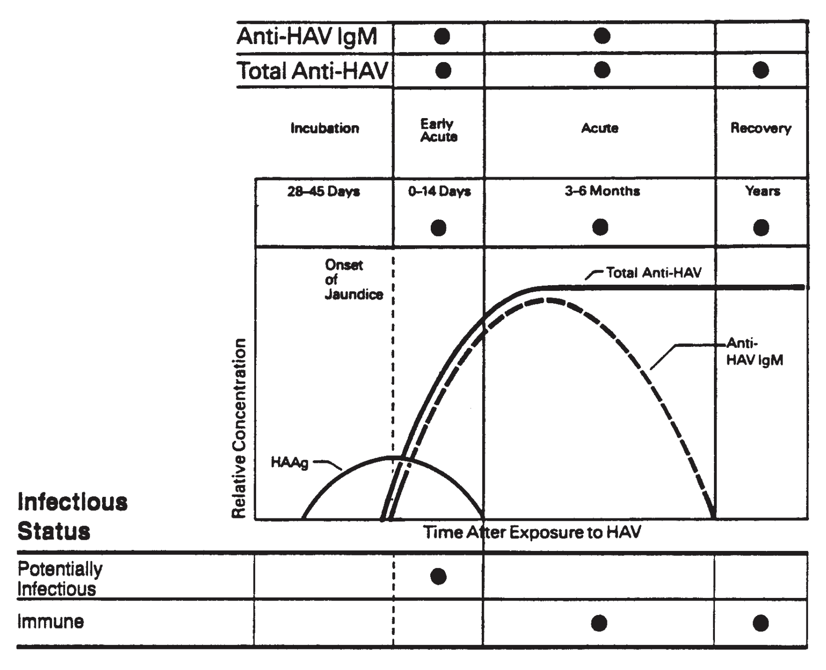


FIGURE 15-5. Temporal relationships of serologies for type A hepatitis with onset of jaundice and infectious status. Anti-HAV IgM is the IgM antibody against the hepatitis A virus. HAAg is the hepatitis A antigen (virus). Total anti-HAV is primarily IgG antibodies (and some IgM in acute phase) against hepatitis A virus. *Source:* Adapted with permission from educational material of Abbott Laboratories, North Chicago, IL.

months.[54] Total antibody, which is comprised of antibody of all isotypes against HAV, indicates present or previous infection or immunization (**Figure 15-5**).

Type B Hepatitis

Hepatitis B virus (HBV) is a DNA virus spread by bodily fluids, most commonly as a sexually transmitted disease, but also via contaminated needles (as with drug abuse or needle stick accidents), shared razor blades or toothbrushes, nonsterile tattooing or body piercing, blood products, or vertical transmission (transmission from mother to child, generally at birth). This disease is 50–100 times more contagious than HIV. The incubation period of HBV varies from two to four months, much longer than that of HAV. Geographically, there is a markedly increased prevalence of hepatitis B in Southeast Asia, China, and sub-Saharan Africa with 10–20% of the populations being hepatitis B carriers. In contrast, the incidence of hepatitis B carriers in the United States is approximately 0.5%.

The clinical illness is generally mild and self-limited but can be quite severe. Unfortunately, up to 5% of infected adults and 90% of infected neonates develop a chronic illness. Chronic HBV infection is often mild, but may progress to cirrhosis, liver failure, or hepatocellular carcinoma, thereby contributing to premature death in 15–25% of cases.[55]

Viral Antigens and Their Antibodies

Three HBV antigens and antibody systems are relevant to diagnosis and management: surface antigen (HBsAg), core antigen (HBcAg), and antigen (HBeAg). HBsAg is present on the outer surface of the virus, and neutralizing hepatitis B surface antibodies (anti-HBs) directed against this protein

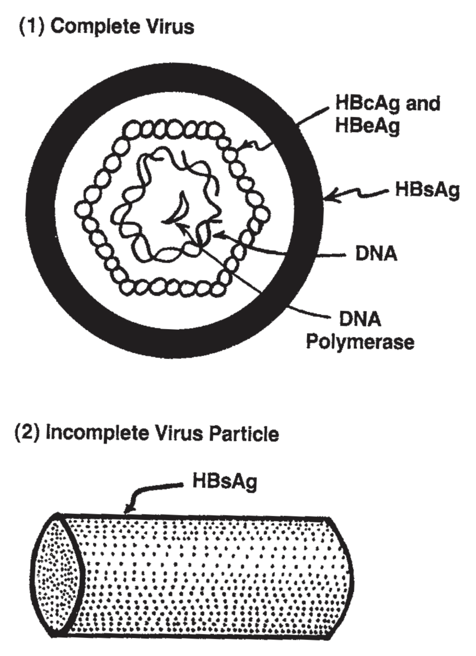


FIGURE 15-6. Hepatitis B virus and its antigenic components. The complete and infectious virus (1), originally known as the Dane particle, is composed of the outer layer (HBsAg) and inner nucleocapsid core. The inner core is comprised of HBcAg intermeshed with HBeAg and encapsulates the viral DNA. HBeAg may be an internal component or degradation product of the nucleocapsid core. An incomplete and noninfectious form (2) is composed exclusively of HBsAg and is cylindrical in shape.

are central to natural and vaccine-induced immunity (**Figure 15-6**). Neither HBcAg nor HBeAg are on the surface of the virion, and thus antibodies against these antigens are not protective. Nevertheless, antibodies are directed against these proteins and may serve as markers of infection. Of these antigens, only HBsAg and HBeAg can be detected in the serum by conventional techniques.[55] HBsAg is detected for a greater window of time during infection and reveals active infection. Detection of HBeAg indicates large amounts of circulating hepatitis B virus; these patients are 5–10 times more likely to transmit the virus than are HBeAg negative persons. HBsAg levels are often used to determine a given patient's suitability for hepatitis B therapy and, subsequently, to monitor for effectiveness of hepatitis B therapy.

TABLE 15-7. Interpretation of Common Hepatitis B Serological Test Results

HBsAg	ANTI-HBs	ANTI-HBc	INTERPRETATION
Positive	Negative	Positive	Acute infection or chronic hepatitis B
Negative	Positive	Positive	Resolving hepatitis B or previous infection
Negative	Positive	Negative	Resolving or recovered hepatitis B or patient after vaccination

anti-HBc = hepatitis B core antibody; anti-HBs = hepatitis B surface antibody; HBsAg = surface antigen of the hepatitis B virus.

In response to infection with HBV, the body may produce anti-HBs, hepatitis B core antibody (anti-HBc), and hepatitis B e-antibody (anti-HBe). All of these antibodies can be detected in clinical laboratories, and in the case of anti-HBcAg separate tests are available to detect IgM or total antibody (all isotypes). Anti-HBs are associated with resolved type B hepatitis or patients who have responded to vaccination for HBV. Anti-HBc is a bit more challenging to interpret, as it can be seen in acute type B hepatitis, after recovery from type B hepatitis (often in concert with anti-HBs), in chronic infection (often with HBsAg and HBeAg), and there can be false-positive results as well. As shown in **Table 15-7** and **Figure 15-7**, levels of antigens and antibodies show complex patterns in the course of HBV infection and thus can yield considerable information about the infection's course and chronology (Table 15-7).

In addition to serological tests, sensitive molecular assays may be used to detect HBV DNA, revealing active viral replication in either acute or chronic infection.[57] These assays may be useful for early detection, as in screening blood donors, because DNA is detectible an average of 25 days before seroconversion.[49] Additionally, some assays allow the quantification of serum viral load, which may be used in the decision to treat and, subsequently, monitor therapy. Presently eight genotypes

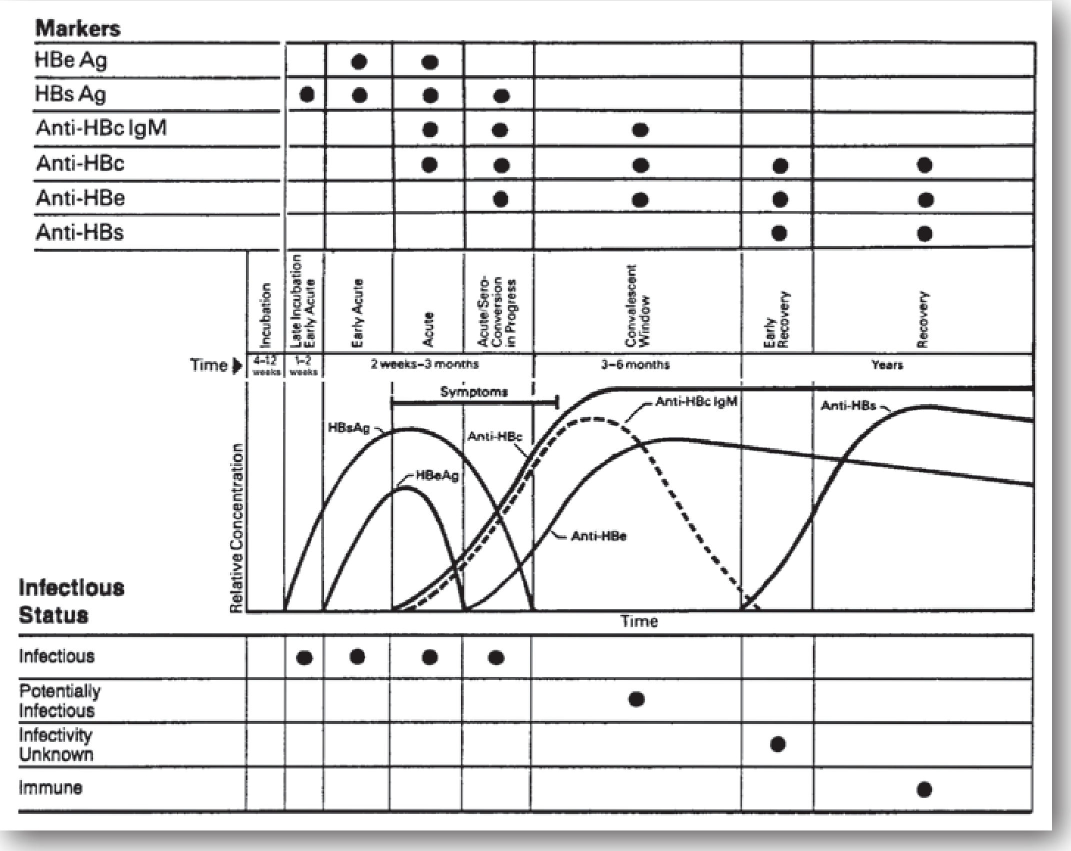


FIGURE 15-7. Serological profile, including temporal relationships integrated with infectious status and symptoms, in 75–85% of patients with acute type B hepatitis. *Source:* Adapted with permission from educational material of Abbott Laboratories, North Chicago, IL.

of HBV have been identified and identification of these genotypes can be of real value in terms of determining appropriate therapy for chronic infection. For example, genotype A—most prevalent in the United States—seems to respond better to interferon than the others, and patients with this genotype might benefit from starting with interferon as opposed to the oral agents available. Genotype C is more prevalent in Asia. For the details of these assays (PCR, RNA:DNA hybrid capture assay, nucleic acid cross-linking assay, and branched DNA assay), refer to a review by Pawlotsky et al.[58]

Acute Type B Hepatitis

HBsAg titers usually develop within 4–12 weeks of infection and may be seen even before elevation of aminotransferases or clinical symptoms (Figure 15-7). Subsequently, HBsAg levels decline as anti-HBs titers develop, which indicates resolution of the acute symptomatic infection and development of immunity. In between the decline of HBsAg and the rise of anti-HBs, there is often a window when neither is present during which time anti-HBcAg may be used to diagnose infection. IgM anti-HBcAg may be used to reveal acute infection as opposed to a flare of chronic HBV.[57]

Chronic Type B Hepatitis

Chronic hepatitis is defined as persistently elevated LFTs for six months. The development of chronic hepatitis B is suggested by the persistence of elevated LFTs (aminotransferases) and is supported by persistence of HBsAg for more than six months after acute infection. Persistence of HBeAg also suggests chronic infection, but some chronically infected patients produce anti-HBeAg and, subsequently, clear HBeAg well after the acute phase is over (late seroconversion; **Figure 15-8**). Clearance of the HBeAg is associated with a decrease in viral DNA, and some degree of remission in chronic hepatitis B. However, this can be confusing as HBeAg is a precore protein, and in patients infected with certain mutations developed during the course of the disease (precore and core promoter), HBeAg may not be produced. Yet even in the face of anti-HBe, there may be active disease with ongoing fibrosis and development of cirrhosis. Although chronically infected individuals usually lack anti-HBsAg, in some cases low levels of non-neutralizing antibodies may be present. Additionally, low levels of IgM anti-HBcAg may persist.[50]

Hepatitis B Vaccine

The *HBV vaccine* consists of recombinant HBsAg, which is not infectious, and it stimulates the production of protective anti-HBs. Generally, this is a safe vaccine, with efficacy of over 90%. Presently it is recommended as a standard vaccine for neonates. Two different HBV vaccines are available in the United States. With one brand, a total of three doses are required: the first dose is given to newborns at birth or before discharge; the second dose at one to two months of age, and the third dose at six months of age. With the other brand, a total of four doses are required and the whole vaccination series is given over 12 months. In those who were not vaccinated at birth, it is indicated for people at high risk of acquiring type B hepatitis or its complications including neonates of mothers with

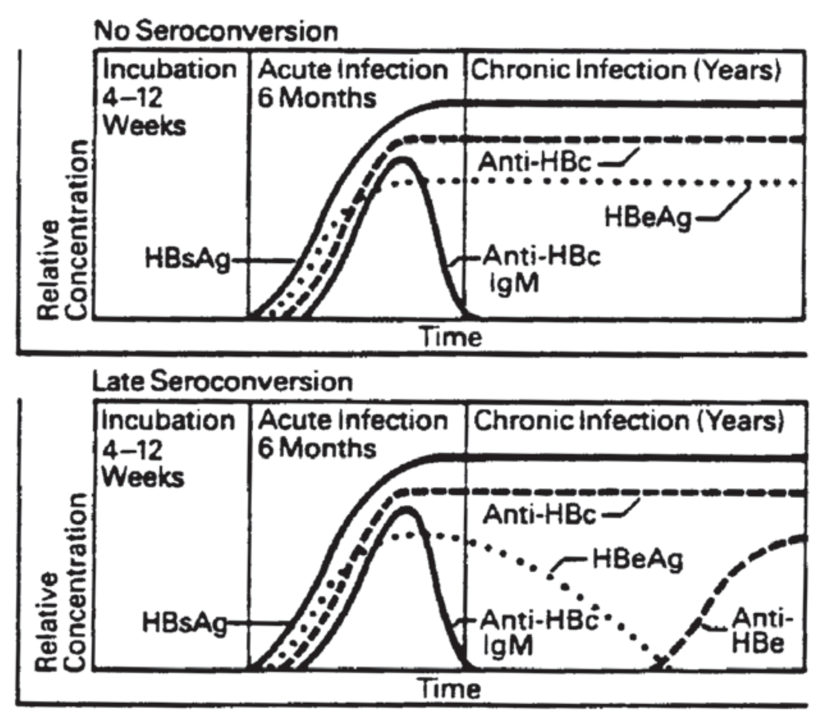


FIGURE 15-8. Serological profiles of patients who chronically carry hepatitis B virus. *Source:* Reproduced with permission from Abbott Laboratories, North Chicago, IL.

hepatitis B, men who have sex with men, injection drug abusers, dialysis patients, healthcare workers, HIV patients, family and household contacts of patients with type B hepatitis, sexually active people with multiple partners, and patients with chronic liver disease. It should also be considered in patients about to undergo chemotherapy or other forms of immunosuppression. More recent efforts, especially in endemic countries, are leading to this being accepted as a universal vaccine. This vaccine, for example, is often required for students in the United States before entering public school.

A vaccine product directed against both HAV and HBV is available. In analyzing serologic data, successful vaccination may be distinguished from previous infection by the presence of anti-HBs and absence of antibodies against other antigens (e.g., HBcAg, HBeAg). Testing for antibodies after vaccination is not generally recommended, with exceptions including healthcare workers, dialysis patients, spouses or sexual partners of infected patients. If these individuals test negative for the antibody to HBV, they should receive a second series of vaccine doses. Although some patients will fail to develop antibodies for a number of reasons, including anergy, these patients should be evaluated for the possibility of occult HBV infection if antibody tests are negative after the second vaccine series. The vaccine has a prolonged duration of action. Routine booster injections are not recommended except, perhaps, for dialysis patients when their titers of anti-HBs are <10 IU/L.

Type C Hepatitis

Hepatitis C virus (HCV) is an RNA virus mainly spread parenterally, although it may also be transmitted vertically and sexually.[56] Although 70–80% of acute infections are asymptomatic, 70–80% of patients develop chronic disease.[51] Given the mildness of the acute attack and the tendency to develop into chronic hepatitis, it is understandable why many patients with this disease first present decades later with cirrhosis or more commonly chronic elevations of aminotransferases. Because chronic HCV infection is often asymptomatic and LFTs may be normal or intermittently elevated, it is recommended that patients at high risk for HCV be screened appropriately. Patients for whom screening would be appropriate include those born in the United States between 1945 and 1965, patients with a history of illegal drug use, including snorting cocaine, as well as parenteral drug abuse. Additionally patients who received clotting factors before 1987 or blood products or organ transplants before July 1992, are HIV positive, have a history of hemodialysis, or have evidence of liver disease (elevated ALT), should be screened.

Acute hepatitis C is often asymptomatic, and when symptoms are present they are mild. Diagnosis of acute hepatitis C, however, is important as evidence suggests that prompt treatment with antiviral medications can prevent progression to chronic hepatitis C in a majority of cases. In chronic hepatitis C infection, the LFTs are usually minimally elevated with ALT and AST values commonly in the 60–100 IU/L range. These values can fluctuate and occasionally return to normal for a year or more, only to rebound when next checked.[59] The primary clinical concern in chronic HCV is that if untreated, within 20 years 20–30% of patients develop cirrhosis and 1–5% develop hepatocellular carcinoma.[51]

The first screening test used is often an enzyme-linked immunosorbent assay (ELISA) assay for anti-HCV, which detects antibodies against a cocktail of HCV antigens. Positive tests can be seen in patients who have passively acquired these antibodies (but not the infection), as a result of blood transfusions, or as children of mothers with hepatitis C. Because of possible cross-reactivity with one of the antigens in the assay, this test has a considerable false-positive rate, and thus positive results need to be confirmed with a more specific assay. One such assay is the recombinant immunoblot assay (RIBA), which is similar to ELISA in principle, but tests antibody reactivity to a panel of antigens individually. Binding to two or more antigens is considered a positive test.[53] Binding to one antigen is considered indeterminate. Presently the approach to a positive ELISA is to skip the RIBA and go directly to the reverse transcriptase polymerase chain reaction (RT-PCR) assay.

Qualitative RT-PCR, often referred to as just *PCR*, detects viral RNA in the blood. It is a very sensitive assay that may be used in diagnosis and subsequent management of hepatitis C. RT-PCR has several advantages compared to serologic tests. It can detect HCV within one to two weeks of exposure and weeks before seroconversion, presentation of symptoms, or the elevation of LFTs. This may be useful because seroconversion only has occurred in 70–80% of patients at the onset of symptoms, and it may never occur in immunosuppressed patients.[59] Additionally there is evidence suggesting that treating acute hepatitis C may be of value. Some immunocompromised patients with hepatitis C (as above) may have false-negative ELISA studies, and thus RT-PCR is recommended for consideration in patients with hepatitis or chronic liver disease who are immunosuppressed. Furthermore, unlike serologic assays, RT-PCR is not confounded by passively acquired antibodies that may be present in uninfected infants or recipients of blood products, and RT-PCR can distinguish between resolved and chronic infection.

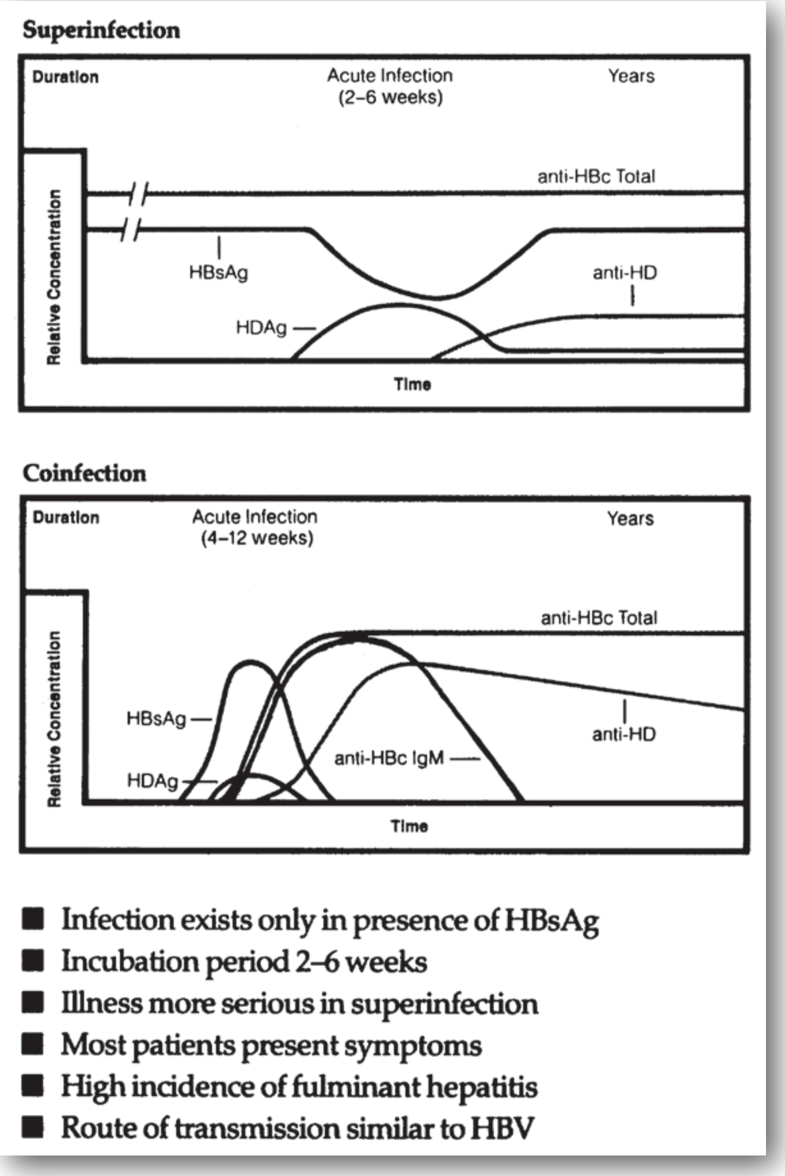
Once a diagnosis of HCV infection is established, various quantitative molecular assays that monitor viral load may be useful in following viral titers during treatment or assessing the likelihood of response to therapy. A major consideration with these tests is that the methodology is not yet standardized, and there is laboratory-to-laboratory variability. These tests are not preferred for initial diagnosis because they are less sensitive than qualitative RT-PCR. They include a quantitative PCR assay and a branched-chain DNA assay (for more information, see Pawlotsky et al.).[58] Presently, most laboratories report HCV PCR measurements in IU/mL, with pretreatment levels often in the millions.

There are at least six major genotypes of the type C virus and multiple subtypes. Viral genotype determination is useful because genotype helps direct the antiviral regimen needed, length of therapy, as well as to predict the likelihood of response to treatment. Recent release of several new oral antiviral agents have improved the treatment of the most common genotype found in the United States (type 1). These newer agents have excluded the use of interferon, shortened the course of treatment, decreased incidence of side effects, and improved success rates. These medicines have also had a similar impact in treating other hepatitis C genotypes, (notably type 2 or 3). Length of treatment may vary depending not only on genotype, presence of cirrhosis, previous exposure to other medications, but on the rapidity of a patient's response to therapy.[60] Genotype determination may be done via direct sequencing or hybridization of PCR amplification products.[59]

Type D Hepatitis

Hepatitis D virus (HDV) is caused by a defective virus that requires the presence of HBsAg to cause infection. Therefore, people only can contract type D hepatitis concomitantly with HBV infection (coinfection) or if chronically infected with HBV (superinfection). Coinfection presents as an acute infection that may be more severe than HBV infection alone.[51] Alternatively, the picture of superinfection is that of a patient, with known or unknown chronic HBV, who develops an acute flare with worsening liver function and increases in HBsAg.[53] Acute coinfection is usually self-limited with rare development of chronic hepatitis, while superinfection becomes chronic in more than 75% of cases and increases the risk of negative sequelae, such as cirrhosis. Transmission of HDV is generally by parenteral routes, although no obvious cause can be determined in some cases.

Testing for HDV is usually only indicated in known cases of HBV infection. The single, widely available assay detects anti-HDV antibodies of all isotypes (**Figure 15-9**). This test is unable to distinguish between acute, chronic, or resolved infection and lacks sensitivity because only about 38% of infected patients have detectible anti-HDV within the first two weeks of illness.[57] Because seroconversion may occur as late as three



Superinfection

Coinfection

- Infection exists only in presence of HBsAg
- Incubation period 2–6 weeks
- Illness more serious in superinfection
- Most patients present symptoms
- High incidence of fulminant hepatitis
- Route of transmission similar to HBV

FIGURE 15-9. Two serological profiles of patients infected with the hepatitis D virus. HDAg = hepatitis D antigen (the virus); anti-HD = antibodies against hepatitis D virus. *Source:* Reproduced with permission from Abbott Laboratories, North Chicago, IL.

months after infection, testing may be repeated if the clinical picture suggests HDV.[50] Tests are also available for HDV RNA, and stains are available to assess the D antigen in hepatocytes.

Type E Hepatitis

Hepatitis E virus (HEV) is generally quite similar to hepatitis A. It is a hardy, protein-coated RNA virus that is spread by the fecal–oral route, often by contaminated food or water. Like HAV, HEV causes an acute illness that is generally self-limited. HEV is endemic in parts of Asia and has become increasingly detected in the United States. Unlike HAV, HEV is notable for a predilection for causing life-threatening illness in women who are in their third trimester of pregnancy. Recently, testing for antibodies to hepatitis E has become available, including HEVAg or hepatitis E antigen. Work is underway to develop a vaccine for hepatitis E.

PRIMARY BILIARY CHOLANGITIS

Primary biliary cholangitis (PBC), previously called primary biliary cirrhosis, is a chronic disease involving progressive destruction of small intrahepatic bile ducts leading to cholestasis and progressive fibrosis over a period of decades. It can progress to cirrhosis and liver failure, necessitating transplantation. Ninety percent of affected individuals are female, with onset occurring between the early twenties and late eighties.[61,62] The etiology of the disease is unknown, although it seems to involve an autoimmune component and has associations with a variety of autoimmune disorders including Sjögren syndrome, rheumatoid arthritis, and scleroderma. The initial symptoms of the disease are often those of progressive cholestasis with fatigue, pruritus, jaundice, and deficiencies in fat-soluble vitamins.[62]

The most useful laboratory test in diagnosing PBC is the detection of antimitochondrial antibodies, with a sensitivity of 95%.[63] This assay is also highly specific, although patients with autoimmune and drug-induced hepatitis occasionally have low antibody titers. PBC usually presents with a predominantly cholestatic laboratory picture, initially with an elevated ALP and GGT, and later, with an elevated total bilirubin. Aminotransferases tend to be minimally elevated or normal.

HEMOCHROMATOSIS

Hemochromatosis is an iron overload state involving the liver and other organs. If left untreated, hemochromatosis can lead to cirrhosis, cardiac failure, diabetes, and hepatocellular carcinoma. Its presentation is often subtle, and most cases are discovered either in patients undergoing evaluation of abnormal hepatic transaminases or presenting with a positive family history of hemochromatosis. Iron overload can be caused either by a primary or secondary disorder. Hereditary hemochromatosis (also referred to as *classical or primary hemochromatosis*) is inherited in an autosomal recessive fashion and involves dysregulated handling of iron absorption from the GI tract.[64] Up to 90% of affected individuals have inherited two alleles carrying the mutant C282Y genotype on chromosome 6.[65] Persons of northern European descent have the highest risk with a greater likelihood in males.[66] Secondary hemochromatosis is generally the result of iatrogenic iron overload from repeated blood transfusions used as therapy for disorders such as thalassemias, sideroblastic anemias, myelodysplastic syndromes, and congenital dyserythropoietic anemias.[67]

Hemochromatosis remains an underdiagnosed disease entity. Many patients report nonspecific symptoms leading to a delay in diagnosis and treatment for up to several years.[68] The classically reported clinical manifestations of hemochromatosis included the triad of bronze skin, diabetes, and cirrhosis but were infrequent at the time of disease diagnosis.[69] Now more commonly patients experience malaise, fatigue, arthralgias, hepatomegaly, and elevated aminotransferase levels when diagnosis is confirmed.

MINICASE 1

A Case of a Prolonged INR and Low Serum Albumin

Jane M., a 50-year-old female, presents to her physician complaining of increasing fatigue and a 20-lb weight loss over the past four months. Initial evaluation shows an albumin of 2 g/dL and an INR of 2.3. Jane M. is referred for evaluation of possible cirrhosis. On further questioning, she denies any history of hepatitis, exposure to hepatotoxins, alcohol use, family history of liver disease, or liver disease.

Jane M.'s physical examination does not suggest liver disease; there is no evidence of ascites, palmar erythema, asterixis, hepatomegaly, splenomegaly, or spider angiomata. It is noted that she has pedal edema. Liver function studies are otherwise normal: ALT 12 IU/L, AST 20 IU/L, total bilirubin 1 mg/dL, and ALP 56 IU/L).

An IM dose of vitamin K 10 mg corrects the INR within 24 hours. Workup shows that Jane M. has malabsorption due to sprue, a disease of the small bowel. With proper dietary management, her symptoms resolve and she gains weight. At a follow-up visit three weeks later, her albumin concentration is 3.7 g/dL, and her edema has resolved.

QUESTION: Why did Jane M. develop a low albumin and a prolonged PT? What caused her pedal edema?

DISCUSSION: This case demonstrates that although low albumin and a prolonged PT suggest advanced liver disease, other causes need to be considered. Administration of vitamin K promptly corrected Jane M.'s INR, suggesting malabsorption of vitamin K. If she had cirrhosis, her PT would not have corrected with the vitamin K. Similarly, her hypoalbuminemia was not due to her liver's inability to synthesize albumin but to the malabsorptive disorder that was interfering with protein absorption. Therefore, Jane M. had a low albumin and elevated INR in the absence of liver disease. Her pedal edema was due to hypoalbuminemia secondary to malabsorption.

Diagnosis typically begins with laboratory assessment of serum ferritin and transferrin iron saturation levels in patients found to have chronically elevated liver enzymes and clinical suspicion of hemochromatosis.[70] A transferrin iron saturation level of over 60% in men or 50% in women may suggest this, although the American Association for the Study of Liver Diseases guidelines suggests 45% as a cutoff. Ferritin levels tend to be elevated as well, over 200 ng/mL in men and 150 ng/mL in women. Hemochromatosis gene testing can then be performed on these patients or individuals at risk for hereditary hemochromatosis based on family history. This can be suggested in patients who are homozygous for the C282Y gene, or compound heterozygote with a copy of the C282Y gene and a copy of the H63D gene. Early diagnosis of hereditary hemochromatosis is important before end organ involvement is evident and is associated with improved outcomes.[71] Therapeutic phlebotomies to decrease serum ferritin levels may result in a normal life expectancy. Management of secondary hemochromatosis is usually through chelation therapy, but life expectancy may be shorter and related to the need for continued transfusion therapy to treat the primary disorder.[72] (**Minicases 1 through 10.**)

MINICASE 2

Jaundice Due to Oral Contraceptives

Amber S., a 16-year-old female, is found by her pediatrician to be slightly jaundiced during a routine school physical. She denies any history of liver disease, abdominal pain, illicit drug abuse, alcohol use, or abdominal trauma. Laboratory evaluation shows a moderately elevated bilirubin of 2.3 mg/dL along with ALP and GGT concentrations of about four times normal. Her AST is 23 IU/L.

Amber S. denies being on any medications (except for vitamins), or being exposed to toxins. Nothing suggests the possibility of a neoplastic or infectious process (temperature of 98.9 °F and WBC count of 7.5 × 10^3 cells/mm^3). Ultrasound of the liver and biliary system is normal with no evidence of biliary dilation.

Her parents take her to a pediatric hepatologist. After much discussion (and threat of a liver biopsy), Amber S. tearfully reveals that she had gone to a local family planning clinic and is using birth control pills.

QUESTION: How might oral contraceptives cause a cholestatic picture? What is the importance of the ultrasound? What is the usual outcome of patients who develop jaundice while taking oral contraceptives?

DISCUSSION: This case demonstrates that oral contraceptives, primarily because of their estrogen content, can cause alterations in cholestatic test results (manifested by an elevated bilirubin, GGT, and ALP) with relatively normal aminotransferases. The ultrasound helps to distinguish between intrahepatic and extrahepatic cholestasis. The absence of biliary dilation suggests intrahepatic cholestasis. The normal AST suggests that jaundice is not due to hepatitis.

Cholestasis from oral contraceptives is generally benign and reverses promptly when the medication is withdrawn. Patients often omit mentioning their use of birth control pills.

MINICASE 3

A 36-Year-Old Executive with Abnormal LFTs

Dana D., a 36-year-old executive, is referred to a prominent medical center for a second opinion. Her physician finds an elevated AST of 180 IU/L on a routine screening exam. Dana D. has no symptoms; her physical examination has been normal, without any signs of liver disease or hepatomegaly.

Additional studies show an ALT of 60 IU/L, a markedly elevated GGT of 380 IU/L, and a minimally elevated ALP of 91 IU/L. Her WBC count is elevated at 20 × 10^3 cells/mm^3. After much discussion, she reveals that she has been drinking 1 pint of vodka a day.

Dana D. enrolls in Alcoholics Anonymous and stops drinking. Three months later, all of her tests are normal: ALT 28 IU/L, GGT 54 IU/L, and ALP 54 IU/L.

QUESTION: What findings suggest alcoholic liver disease? Why did all of Dana D.'s laboratory test results return to normal? What else might have happened in this situation?

DISCUSSION: This case demonstrates several aspects of alcoholic liver disease. The diagnosis is suggested by an elevated AST out of proportion to the ALT, as well as by a markedly elevated GGT with a normal (or virtually so) ALP. An elevated MCV, if present, also would support this diagnosis. Patients with alcoholic liver disease may have markedly elevated WBC counts.

Alcoholic liver disease tends to have several different stages. The earliest manifestation may be just a "fatty liver," which is generally reversible with cessation of alcohol intake. Alcoholic hepatitis and cirrhosis can follow with excessive alcohol intake. Unfortunately, alcoholic cirrhosis can develop without any warning signs. If Dana D. had alcoholic cirrhosis, stopping alcohol consumption probably would not have significantly altered her abnormal test results.

Clinicians should remember that a patient does not need to be a "skid row" alcoholic to develop alcoholic cirrhosis. Women are more susceptible to the hepatotoxic effects of alcohol than are men, and as few as two or three drinks a day can cause significant liver disease in susceptible persons, this being due to differences in alcohol metabolism.

MINICASE 4

A Jaundiced College Student

Jacob N., a 19-year-old college student, anxiously reports to the infirmary when his girlfriend notices that he has become yellow. He feels well and has a normal physical examination. On discussion, he indicates that he has recently embarked on a rigorous crash diet in anticipation of winter break in Florida.

The evaluation shows an elevated total bilirubin of 4.8 mg/dL, of which 90% was unconjugated (4.3 mg/dL). The absence of hemolysis is established by microscopic examination of a blood smear, normal reticulocyte count, and LDH, which is 112 IU/L. Jacob N.'s other LFTs are normal: ALT 21 IU/L and ALP 76 IU/L.

QUESTION: What was the most likely cause of Jacob N.'s signs and symptoms? How should his condition be managed? What is his prognosis?

DISCUSSION: Elevated bilirubin concentrations do not necessarily indicate severe liver disease. The normal ALT and ALP rule out hepatocellular and cholestatic liver diseases. If done, AST would have been normal. The normal LDH, RBC microscopic exam, and reticulocyte count rule out hemolysis as a cause of the elevated bilirubin. The normal LDH is also consistent with a lack of intrinsic liver disease.

Jacob N. should be reassured that he has Gilbert syndrome and might become somewhat jaundiced with fasting or acute or chronic illness. Gilbert syndrome is not associated with any symptoms, is totally benign, and requires no treatment. When a patient has an elevated bilirubin, a practitioner should always obtain LFTs before providing a diagnosis or performing unnecessary tests.

MINICASE 5

Hepatic Encephalopathy

Stephen F., a 47-year-old alcoholic, is admitted to a hospital after being found on a park bench surrounded by empty beer bottles. Known to have cirrhosis, Stephen F. is thought to be showing signs of hepatic encephalopathy as he slowly lapses into a deep coma over the first four days of hospitalization. His physical examination is significant in that he has hepatomegaly and splenomegaly.

Laboratory evaluation shows a negative urine drug screen for central nervous system (CNS) depressants with serum glucose mildly elevated at 120 mg/dL. All serum electrolytes are normal: sodium, 140 mEq/L; potassium, 4 mEq/L; chloride, 98 mEq/L; carbon dioxide, 25 mEq/L; and magnesium, 1.5 mEq/L. Stephen F.'s blood alcohol concentration on admission is 150 mg/dL (normal: 0 mg/dL). His serum GGT is 321 IU/L, and his AST is 87 IU/L.

Unfortunately, efforts at treating hepatic encephalopathy do not reverse his coma. Further examination and testing are undertaken when it is noted that his ammonia concentration are normal at 48 mcg/dL. Then, a large bruise is noticed on the side of Stephen F.'s head, and a CT scan reveals a large subdural hematoma. With surgical treatment of the hematoma, he promptly awakes and began asking for more beer.

QUESTION: How does one establish the diagnosis of hepatic encephalopathy for this patient? What is the role of the serum ammonia concentration in the diagnosis?

DISCUSSION: This case demonstrates that the diagnosis of hepatic encephalopathy is not always straightforward. Hepatic encephalopathy is only one cause of altered mental function in patients with advanced liver disease. Other causes may include accumulation of drugs with CNS depressant properties, head trauma, hypoglycemia, delirium tremens, and electrolyte imbalances. The diagnosis of hepatic encephalopathy is suggested by the following:

- Elevated ammonia concentrations
- Presence (in early stages) of asterixis or a flapping tremor of the hands
- Absence of other causative factors
- Characteristic electroencephalographic findings (rarely used)

The response to therapy (usually correction of electrolyte imbalances, rehydration, and lactulose or rifaximin) further supports this diagnosis. Serum ammonia concentrations, therefore, are just one piece of this puzzle. An elevated concentration suggests, but does not establish, this diagnosis. Furthermore, although normal ammonia concentrations may cause one to question the diagnosis of hepatic encephalopathy, they can occur in this condition.

MINICASE 6

Laboratory Diagnosis of Acute Hepatitis

Michael C., a 45-year-old executive, presents to his physician after noticing that he has been turning yellow. Other than increased fatigue, he says he feels well. His physical examination is normal except for his jaundice and tenderness over a slightly swollen liver. Initial laboratory studies show elevations of the aminotransferases with an ALT of 1235 IU/L and an AST of 2345 IU/L. His total bilirubin is 18.6 mg/dL.

The tentative diagnosis is acute hepatitis. However, Michael C. has not had any transfusions, used parenteral drugs, or had recent dental work. No exposure to medications or occupational exposure accounts for the disease, and there is no family history of liver disease. Careful review of his history offers no explanation for his development of hepatitis.

Ultimately, serologies show a positive HBsAg and anti-HBc antibody. A diagnosis of acute type B hepatitis is established. After much questioning, Michael C. reveals that he had a "brief encounter" with a prostitute on a recent business trip. His wife is treated with the vaccine and hepatitis B immunoglobulin, a γ-globulin with high concentrations of antibodies to HBsAg, and she does not develop hepatitis.

QUESTION: How is this diagnosis established? How should Michael C. be followed, and what is the likely outcome?

DISCUSSION: This case demonstrates why a determination of the etiology of hepatitis is often difficult. The practitioner must obtain a detailed history of exposures to medicines, drugs, alcohol, infected people, family members with similar illness, and ongoing medical illness. In this case, the exposure to the prostitute put Michael C. at risk for hepatitis B, C, and D and for HIV. The diagnosis is established by the serologies. If just the anti-HBc antibody been present, he could have

- been in the "window" phase of the acute disease where this antibody is positive and HBsAg is negative
- previously recovered from hepatitis B
- been a chronic carrier

Although the additional presence of HBsAg helps to secure the diagnosis, the clinical picture must be considered. Both HBsAg and anti-HBc also can be positive in patients with chronic hepatitis B. Determination of the type of hepatitis has prognostic value and, in this case, allows administration of prophylactic medications to people who might have been exposed.

HBsAg	ANTI-HBs	ANTI-HBc	INTERPRETATION
Positive	Negative	Positive	Acute infection or chronic hepatitis B
Negative	Positive	Positive	Resolving hepatitis B or previous infection
Negative	Positive	Negative	Resolving or recovered hepatitis B or patient after vaccination

There is no generally accepted drug therapy for type B acute viral hepatitis. Michael C. should have repeated physical examinations and repeat laboratory testing for albumin, INR, AST, ALT, and bilirubin. There is a >1% chance that he will develop fulminant hepatitis and die. Most likely, he will recover completely, with his LFTs normalizing over one to two months. However, there is a 10–20% chance that he will develop chronic hepatitis, which could lead to cirrhosis.

MINICASE 7

Laboratory Diagnosis of Hepatitis Type C

Katherine M., a 48-year-old female, receives a notice two weeks after donating blood that it could not be used because her aminotransferases are elevated and a test for hepatitis C is positive. She is referred to a specialist; her ALT is 87 IU/L, her AST is 103 IU/L, and her bilirubin is 0.8 mg/dL.

A liver biopsy demonstrates chronic active hepatitis. After considerable discussion, Katherine M. is placed on oral drug therapy for hepatitis C. After three months of therapy, however, her aminotransferases are not responding, and she is referred for a second opinion.

Type C hepatitis is excluded when her laboratory results are repeated, and she is found to have negative titers to detect HCV RNA (PCR). Additional history is then obtained. Katherine M. reluctantly tells her local family physician that, about six months previously, she had a positive skin test for tuberculosis (TB) after discovering that her partner is HIV positive. She also says she sought treatment in a nearby city, had been placed on isoniazid, and finished the prescription but never returned to the clinic.

QUESTION: What are the roles of the second-generation and third-generation tests in the diagnosis of hepatitis C? What other nonviral forms of hepatitis can present as chronic hepatitis?

DISCUSSION: This case demonstrates several points. Many patients with chronic active hepatitis remain totally asymptomatic—their disease first being detected during routine blood work or when symptoms of cirrhosis or liver failure develop. Now that all blood donors are checked for hepatitis C and abnormal aminotransferases, many patients with chronic liver disease are detected before symptoms are present. Unfortunately, however, blood banks tend to use the less expensive and less accurate ELISA testing for hepatitis C, which (as in this case) gives many false-positive results. Before starting therapy or establishing a diagnosis of type C hepatitis, a practitioner should confirm the diagnosis with RT-PCR.

A similar picture also may be seen in autoimmune hepatitis, Wilson disease, α1-antitrypsin deficiency, and hemochromatosis. Isoniazid, a common medication for TB, can cause serious liver damage that may be clinically and histologically indistinguishable from viral chronic active hepatitis. Therefore, aminotransferases need to be carefully monitored in patients on this drug. This problem tends to occur in patients over the age of 50, particularly women.

The aminotransferases may only be minimally elevated, as in this patient. Generally, hepatitis develops after about two to three months of drug therapy. With withdrawal of the medication, the numbers usually return to normal over an additional one to two months. When the diagnosis of chronic active hepatitis is considered, a patient's medications must be very carefully reviewed.

MINICASE 8

A Case of Acute Liver Failure

Matthew Z., an 18-year-old high school senior, notices he is becoming increasingly tired and somewhat weak. He initially is taken to a local clinic where he is reassured that most likely he is getting the "flu" and is sent home. Over the next day, he becomes progressively weaker, and his family notices that he is becoming yellow. He is taken to his physician.

He denies being on any medications or supplements. There is no family history of liver disease. Matthew Z. denies alcohol use. There has been no recent history of unusual travel.

Initial physical examination is unremarkable except for his being markedly icteric (jaundiced) and his liver being somewhat enlarged.

Laboratory findings include ALT 1354 IU/L, AST 1457 IU/L, and total bilirubin 12.5 mg/dL. Serum ALP is 245 IU/L.

The tentative diagnosis is acute viral hepatitis. However, as more laboratory results come back, this diagnosis is increasingly under question because they show negative serologies for hepatitis A, B, C, and E. Evaluation for autoimmune hepatitis is negative as well with a negative ANA (antinuclear antibody) and ASMA (anti-smooth muscle antibody). Serologies for mononucleosis and cytomegalovirus (CMV) are negative as well.

Matthew Z. is admitted to the hospital and over the next two days his total bilirubin progressively climbs to 23.4 mg/dL, and his INR increases to 4. He becomes increasingly confused and lethargic, and a serum ammonia level is found to be elevated at 348 mcg/dL.

A diagnosis of acute liver failure is made, and he is transferred to a liver transplant center where he admits that in the days before the onset of his illness, he had visited his girlfriend in college and they both took some ecstasy (MDMA, 3,4-methylenedioxy-N-methylamphetamine).

QUESTION: What is acute liver failure and what generally causes it?

DISCUSSION: Acute liver failure refers to the rapid development (typically within eight weeks) of severe liver injury with associated failure of the liver to perform its usual synthetic/detoxifying functions. In this case, it is evident by the elevated bilirubin, prolonged INR, and the development of hepatic encephalopathy. There are many potential causes, such as drug or toxin related, with acetaminophen being among the most common culprits. Viral hepatitis can cause this picture as can ischemia of the liver, sepsis, autoimmune hepatitis, malignancy, and certain conditions associated with pregnancy.

MDMA hepatotoxicity is increasingly common in young people, as this drug is increasingly used. It can cause subclinical liver damage including fibrosis and is rarely associated with a picture of fulminant liver failure as in Matthew Z. Treatment is largely supportive and may involve liver transplantation.

MINICASE 9

A Case of Drug-Induced Hepatotoxicity

Darcie D. is a 43-year-old executive who presents to her doctor because she is not feeling well. She has become increasingly fatigued, lost her appetite, and just feels "sick."

Her past medical history is generally unremarkable; she does not smoke, drinks only socially, and does not take any medications or supplements. Physical examination is entirely normal.

Screening laboratory tests include a normal CBC, BUN, serum creatinine, and serum electrolytes. LFTs, however, are remarkable: ALT 257 IU/L and AST 397 IU/L. Her bilirubin is elevated at 2.3 mg/dL, and alkaline phosphatase is elevated at 367 units/L.

Additional laboratory findings include negative serologies for hepatitis A, B, and C. Tests to rule out autoimmune hepatitis (ANA, anti-smooth muscle antibody) are negative, and tests to exclude hemochromatosis (iron, TIBC, and ferritin) are also normal.

Darcie D. presents again to her doctor's office, and on further questioning, she says that three weeks before, while on a business trip to California, she developed a bad cold and had been given azithromycin "Z-pak" at a local clinic. Over the next two weeks, Darcie's D.'s laboratory results gradually return to normal, and she reports feeling better.

QUESTION: Is it common for patients to develop symptomatic hepatitis weeks to months after exposure to hepatotoxins?

DISCUSSION: When we think of hepatotoxic agents (drugs, supplements, toxins) we tend to limit our thinking to the patient's current exposures. Usually, this alone solves our diagnostic dilemma. It is useful, however, to realize that often hepatoxicity can present weeks (occasionally months) after exposure. One classic agent that can present this way is amoxicillin–clavulanate. Azithromycin can present with jaundice, abdominal pain, pruritus, and evidence of hepatocellular injury weeks after a brief exposure. Treatment is generally supportive.

PANCREATIC INFLAMMATION/ PANCREATITIS

Pancreatitis describes inflammation of the pancreas (either acute or chronic) and is the most common disease associated with this gland. Although there are multiple causes of pancreatitis, the clinical presentation is often the same. Acute pancreatitis generally presents with severe midepigastric abdominal pain developing over an hour, often radiating to the back. The pain tends to be continuous and can last for several days.

This condition is often associated with nausea and vomiting; in severe cases, fever, ileus, and hypotension can occur. Ultimately, there can be progressive anemia, hypocalcemia, hypoglycemia, hypoxia, renal failure, and death. The clinician faces the challenge of rapidly establishing this diagnosis, because many conditions (e.g., ulcers, biliary disease, myocardial infarction, and intestinal ischemia or perforation) can present in a similar manner. Most individuals with acute pancreatitis can make full recoveries; however, the long-term inflammation associated with chronic pancreatitis can lead to fibrosis and calcification of the pancreas causing irreversible damage, occasionally leading to the development of diabetes mellitus or malabsorption due to deficiencies in the production of pancreatic enzymes.

Gallstones and alcohol abuse are causative factors in 60–80% of acute pancreatitis cases.[73] Medications can also cause acute pancreatitis (**Table 15-8**). Other possible causes include autoimmune diseases, trauma (a typical example being an injury due to a bicycle handlebar), penetrating ulcers, hypercalcemia, hypertriglyceridemia, pancreatic neoplasm, and hereditary or autoimmune pancreatitis. Often, however, it is impossible to determine the definite cause of a patient's attack. The tests discussed in this section, amylase and lipase, are primarily used to diagnose pancreatitis, although they may be clinically useful in the diagnosis of other pathologies.

TABLE 15-8. Selected Drugs That May Cause Pancreatitis[a]

- 5-ASA drugs
- ACE inhibitors
- Anti-viral medications
- Asparaginase
- Atypical antipsychotics
- Azathioprine
- Cimetidine
- Corticosteroids
- Didanosine
- Estrogens
- Exenatide
- Furosemide
- Isoniazid
- Mercaptopurine
- Methyldopa
- Metronidazole
- Nitrofurantoin
- Pentamidine
- Ranitidine
- Sitagliptin
- Statins
- Sulfonamides
- Sulindac
- Tetracycline
- Thiazides
- Valproic acid

ACE = angiotensin-converting enzyme; ASA = acetylsalicylic acid.

[a] A good review on drug induced pancreatitis, with a much more inclusive list is seen in Jones et al.[74.]

Amylase

Normal range: 20–96 units/L (method dependent)
(0.34–1.6 μkat/L)

Amylase helps break starch into its individual glucose molecules. The enzyme's most frequent clinical use is in the diagnosis of acute and chronic pancreatitis. Although amylase levels are often used for this diagnosis, increasingly lipase (see below) is preferred in part due to the longer half-life of the latter.

As with any serum protein, concentrations result from the balance between entry into circulation and rate of clearance. Most circulating amylase originates from the pancreas and salivary glands. These sources are responsible for approximately 40% and 60% of serum amylase, respectively. However, the enzyme is also found in the lungs, liver, fallopian tubes, ovary, testis, small intestine, skeletal muscle, adipose tissue, thyroid, tonsils, and certain cancers, and various pathologies may increase secretion from these sources. The kidneys are responsible for about 25% of the metabolic clearance, with the remaining extrarenal mechanisms being poorly understood. The serum half-life is between one to two hours.[63,75,76] Patients with azotemia can have decreased amylase clearance and elevated amylase levels. More than half of the patients who have a creatinine clearance between 13–39 mL/min have elevated amylase levels.[77] Although there is no amylase activity in neonates and only small amounts at two to three months of age, concentrations increase to the normal adult range by one year of age.

Amylase concentrations rise within 2–6 hours after the onset of acute pancreatitis and peak after 12–30 hours if the underlying inflammation has not recurred. In uncomplicated disease, these concentrations frequently return to normal within three to five days. More prolonged, mild elevations occur in up to 10% of patients with pancreatitis and may indicate ongoing pancreatic inflammation or associated complications (e.g., pancreatic pseudocyst).

Although serum amylase concentrations do not correlate with disease severity or prognosis, a higher amylase may indicate a greater likelihood that the patient has pancreatitis.[78] For example, serum amylase concentrations may increase up to 25 times the upper limit of normal in acute pancreatitis, while elevations from opiate-induced spasms of the sphincter of Oddi generally are <2–10 times the upper limit of normal. Unfortunately, the magnitude of enzyme elevation can overlap in these situations, and ranges are not very specific.

Amylase has a relatively low sensitivity, with about 20% of patients with acute pancreatitis having normal levels. This is especially common in patients with alcoholic pancreatitis or pancreatitis due to hypertriglyceridemia. Additionally, amylase has relatively low specificity and may be elevated in a wide range of conditions. These include a variety of diseases of the pancreas, salivary glands, GI tract (including hepatobiliary injury, perforated peptic ulcer, and intestinal obstruction or infarction), and gynecologic system (e.g., ovarian or fallopian cysts), as well as pregnancy, trauma, renal failure, various neoplasms, and diabetic ketoacidosis. Additionally, alcohol and a variety of medications including, but not limited to, aspirin, cholinergics, thiazide diuretics, and oral contraceptives may also cause increased values.[79] In diagnosing acute pancreatitis, other useful laboratory tests include lipase because it is confounded by fewer factors, and fractionation of serum amylase into pancreatic and salivary isoenzymes (although its utility has been questioned).[80,81]

Another condition that may cause elevated amylase concentrations is macroamylasemia, a benign condition present in 2–5% of patients with hyperamylasemia.[82] In this condition, amylase molecules are bound by immunoglobulins or complex polysaccharides, forming aggregates that are too large to enter the glomerular filtrate and be cleared by the kidneys. This results in serum concentrations up to 10 times the normal limit.[83] Macroamylasemia can be detected by fractionating serum amylase or by measuring urine amylase.

Urine amylase concentrations (normal range: <32 IU for a two-hour collection or <384 IU for a 24-hour collection) usually peak later than serum concentrations, and elevations may persist for 7–10 days. This is useful if a patient is hospitalized after acute symptoms have subsided at which point serum amylase may already have returned to normal, leaving only urinary amylase to indicate pancreatitis. As discussed below, lipase may also persist after serum amylase levels decline. Urine amylase levels may also be useful in revealing macroamylasemia, in which case serum amylase is elevated, while urinary amylase is normal or decreased.[81] However, this pattern of elevated serum amylase without elevated urinary amylase is also consistent with renal failure.

One cause of amylase's relatively low sensitivity is that marked hypertriglyceridemia may cause amylase measurements to be artificially low, masking an elevation in serum amylase. This finding is clinically relevant because hypertriglyceridemia (>800 mg/dL) is a potential cause of acute pancreatitis. In this situation, serial dilution of the serum specimen can eliminate the assay interference of hypertriglyceridemia, and elevated amylase values can be identified and measured. Fortunately, urinary amylase and serum lipase would typically be abnormal in this situation and this is another way to assess patients suspected to have elevated serum amylase levels.

Lipase

Normal range: 31–186 units/L (0.5–3.2 μkat/L)

Lipase is an enzyme secreted by the pancreas that is transported from the pancreatic duct into the duodenum where it aids in fat digestion. Lipase catalyzes the hydrolysis of triglycerides into fatty acids and glycerol, simpler lipids that are more readily absorbed and transported throughout the body. Although mostly secreted by the pancreas, lipase can also be found in the tongue, esophagus, stomach, small intestine, leukocytes, adipose tissue, lung, breast milk, and liver. In healthy individuals, serum lipase tends to be mostly of pancreatic origin.[84]

Lipase initially parallels amylase levels in acute pancreatitis, increasing rapidly and peaking at 12–30 hours. However, lipase has a half-life of 7–14 hours so that it declines much more slowly, typically returning to normal after 8–14 days. Thus, one utility of lipase (similar to urinary amylase) is the detection of acute pancreatitis roughly three or more days after onset at which point amylase levels may no longer be elevated. As with amylase, peak lipase concentrations typically range from three to five times the upper limit of the reference range.

A comparison of the sensitivity and specificity of amylase versus lipase, and the utility of these tests alone or in combination is debated. This issue is complicated by the fact that the sensitivity and specificity of any laboratory test vary depending on where the cutoff is chosen (e.g., choosing a higher cutoff increases specificity at the cost of lower sensitivity). In general, serum lipase appears to be superior, particularly with respect to specificity.[80-82,85-87] However, simultaneous determination of both lipase and amylase may increase overall specificity because different factors confound the different assays.[88] For example, an elevated amylase with a normal lipase suggests amylase of salivary origin, or may represent macroamylasemia. Similarly, an elevated lipase with normal amylase has been shown to often not be due to pancreatitis, although in the case of pancreatitis it could be caused by delayed laboratory evaluation or artificial lowering of amylase levels by hypertriglyceridemia.[89]

Lipase concentrations may be elevated in patients with nonpancreatic abdominal pain such as a ruptured abdominal aortic aneurysm, and a variety of disorders of the alimentary tract and liver such as intestinal infarction. This is because lipase is located in these organs. Renal failure, nephrolithiasis, diabetic ketoacidosis, and alcoholism are conditions where lipase elevations tend to be present, however, usually in concentrations less than three times the upper limit of the reference range. Drug-induced elevations in lipase can be attributed to opioids (codeine, morphine), NSAIDs (indomethacin), and cholinergics (methacholine and bethanechol).[89] In the condition of macrolipasemia, similar to macroamylasemia but far less frequent, macromolecular complexes of lipase to immunoglobulin prevent excretion and elevate serum lipase concentrations.[90]

Other Test Results in Pancreatitis

In severe cases of acute pancreatitis, occasionally several days after the insult, fat necrosis may result in the formation of organic soaps that bind calcium. Serum calcium concentrations then decrease (low albumin may also contribute), sometimes enough to cause tetany. When pancreatitis is of biliary tract origin, typical elevations in ALP, bilirubin, AST, and ALT are seen. Some researchers believe that in acute pancreatitis an increase of ALT to three times baseline or higher is relatively specific for gallstone-induced pancreatitis.[91]

Pancreatitis also may be associated with hemoconcentration and subsequent elevations of the BUN or hematocrit. Depending on the severity of the attack, lactic acidosis, azotemia, anemia, hyperglycemia, hypoalbuminemia, or hypoxemia may also occur.

Despite the performance of amylase and lipase assays for acute pancreatitis, the sensitivity and specificity of these tests are often regarded as unsatisfactory, and in some patients pancreatitis only is diagnosed on autopsy. For this reason, several new tests have been investigated (e.g., serum trypsin and trypsinogen), although they are not yet widely available.[87,92] Ultimately, it is recognized that the lack of sensitivity for both amylase and lipase implies that these tests can be used to support a diagnosis of acute pancreatitis but may not definitively provide a secure diagnosis, particularly if the levels are not dramatically elevated.[86] Recent practice guidelines suggest that two of the following are needed to diagnose acute pancreatitis: (1) characteristic symptoms, (2) elevation of amylase or lipase to at least three times normal, and (3) characteristic findings on imaging (usually CT or MRI scanning). (**Minicase 11**.)

ULCER DISEASE

Up to 10% of the U.S. population will develop *ulcers* at some point in life. For many decades ulcers were believed to be primarily due to acid. Traditional therapy with antacids, histamine$_2$-antagonists, and proton pump inhibitors (PPIs) has been effective in treating ulcers, but they are not as effective in preventing recurrences, in part because they do not eradicate the underlying bacterial cause.

H. pylori

H. pylori has been identified as a cause of ulcer disease, and studies into its detection and treatment are still in a state of rapid development. *H. pylori* is a gram-negative bacillus, usually acquired during childhood, which establishes lifelong colonization of the gastric epithelium in affected individuals. Transmission seems to be by the fecal–oral or oral–oral route. Prevalence increases with age and correlates with poor sanitation.[93] By the age of 50, 40–50% of people in developed countries and over 90% of people in developing countries harbor these bacteria.[94]

H. pylori infection may be found in more than 90% of patients with duodenal ulcers and more than 80% of patients with gastric ulcers.[98] Furthermore, the bacterium has been associated with the development of antral gastritis, gastric cancer, and certain types of gastric lymphoma. [95-97] It has not been associated with nonulcer dyspepsia. The most common lymphoma associated with *H. pylori* is MALT (mucosa-associated lymphoid tissue), which is often curable just by treating the underlying *H. pylori* infection. However, most infected individuals (>70%) are asymptomatic, and eradication therapy is not a standard recommendation for asymptomatic colonization.[97] From the other perspective, *H. pylori*-infected individuals have a 10–20% chance of developing peptic ulcers and a 1–2% chance of developing gastric cancer during their lifetime.[99] Candidates for routine screening for *H. pylori* include those with active ulcer disease, history of ulcer disease, and certain gastric lymphomas. Routine screening should be considered prior to long-term therapy with NSAIDs. One problem in managing patients with *H. pylori* infection is that treatment is not always successful in eradicating this bacterium, in part due to increasing resistance to antibiotics. In the United States, rates of resistance to metronidazole (20–40%) and clarithromycin (10–15%) have been documented.[100]

H. pylori-Associated Gastric Cancer

H. pylori-associated gastric cancers account for about 5.5% of all cancers worldwide and about a quarter of all infection-associated cancers.[101] Its colonization is a key component for the development of gastric cancer; however, other factors, such as atrophic changes in the stomach, are needed for this to occur. Atrophic gastritis, characterized by chronic inflammation of the gut mucosa, decreases and ultimately inhibits the ability of the stomach to secrete acid. Eradication of *H. pylori* infection before atrophic changes occur provides almost full protection from gastric cancer.[101] Individuals who have already suffered irreversible atrophic changes will still receive some benefit but should be considered at risk even after eradication. Currently, there is no effective *H. pylori* vaccine available, so bacterial eradication must be executed with antibiotic therapy.

Diagnosis

The diagnostic tests for *H. pylori* are classified as *noninvasive* (serology, urea breath test, and fecal antigen test) or *invasive* (histology, culture, and rapid urea test)—the latter depending on upper endoscopy and biopsy. The serological test for *H. pylori* detects circulating immunoglobulin G antibodies against bacterial proteins. It has a relatively low sensitivity and specificity (80–95%) but has advantages of being widely available and inexpensive.[94,102] Although useful to establish an initial diagnosis of *H. pylori*, it should not be used to monitor the success of eradication therapy as antibody titers decrease slowly in the absence of bacteria.

The urea breath test is based on the ability of the bacteria to produce urease, an enzyme that breaks down urea and releases ammonia and carbon dioxide as its products. In the breath test, ^{13}C- or ^{14}C-labeled urea is given by mouth. If the bacteria are present, the radiolabeled urea is metabolized to radiolabeled CO_2, which may be measured in exhaled air. The tests have high sensitivity and specificity (both 90–95% for ^{13}C and 86–95% for ^{14}C).[94,97] However, the ^{14}C isotope has the drawback of being radioactive, and the ^{13}C isotope requires the use of sophisticated detection methods such as isotope ratio mass spectrometry (although samples are stable and may be mailed away for analysis).[103]

The fecal antigen test detects *H. pylori* proteins in stool via ELISA. It has high sensitivity and specificity, both 90–95%. Like the urea breath test, the fecal antigen test is a very accurate noninvasive measure that is used primarily to monitor the success of eradication therapy. Also, it may be used as a test for infection when endoscopy is not indicated.[104] The fecal antigen test is not appropriate for patients with active GI bleeding because of a cross-reactivity with blood constituents in the immunoassay, which can produce a high incidence of false-positive results.[105] Patients need to discontinue PPIs for two (preferably four) weeks before these test is conducted as PPIs may decrease the numbers of *H. pylori* in the stomach and decrease the accuracy of the tests.

Upper endoscopy with biopsy of gastric tissue and subsequent histological examination has high sensitivity and specificity (88–95% and 90–95%, respectively) with the added advantage of allowing detection of gastritis, intestinal metaplasia, or other histological features. Although not commonly performed, biopsy specimens may also be used to culture *H. pylori*. By performing various tests on the cultured bacteria, this test may be rendered highly specific (95–98%), but the bacterium is difficult to culture making this the least sensitive test (80–90%).[93,94] The main advantage of culture is that it allows for antibiotic sensitivity testing, which can help optimize therapy and possibly prevent treatment failure. A rapid urease test (also known as the *CLO test* or *Campylobacter-like organism test*) involves incubating a biopsy specimen in the presence of urea and a pH indicator. As mentioned above, *H. pylori* metabolizes urea, releasing ammonia, which in this case may be detected by its effect of increasing the pH. This test allows for rapid results (e.g., one-hour incubation time following endoscopy), high sensitivity and specificity (both 90–95% respectively), and low cost.[94,107] One proposed strategy is to take several biopsies at the time of endoscopy and first check the rapid urease test, sending specimens for detailed pathologic analysis only if the urease test is negative (or tissue diagnosis is needed to sort out other diagnoses). One other invasive test requires a nasogastric catheter; PCR detection of *H. pylori* DNA may be performed on gastric juice extracted via the catheter.[93]

All of these tests, with the exception of serology, tend to be confounded by factors that lower bacterial burden. In patients with achlorhydria or patients being treated with antisecretory drugs (e.g., PPIs), increased stomach pH decreases bacterial levels and may lead to false-negative results.[103,106,108,109] Similarly, use of bismuth or antibiotics (including recent, unsuccessful eradication therapy) may decrease test sensitivity. Recommendations advise waiting two to three months after finishing therapy before performing these tests to determine whether or not *H. pylori* has been successfully eradicated and additionally holding PPIs for two to four weeks and antibiotics for four weeks prior to testing.

Although GI bleeding may confound the rapid urease test and the fecal antigen test, urea breath tests remain a viable diagnostic option in patients with active bleeding, detecting 86% of *H. pylori*-positive patients.[104-108] Generally routine testing is not recommended for confirmation of bacterial clearance after therapy. Exceptions include patients with *H. pylori*-induced ulcer, patients not responding clinically, and those with *H. pylori*-associated MALT lymphoma or gastric cancer.

COLITIS

Colitis—acute or chronic inflammation of the colon—often presents quite dramatically with profound and bloody diarrhea, urgency, and abdominal cramping. It is generally distinguished from noninflammatory causes of diarrhea on the basis of physical signs including fever, abdominal tenderness, and an elevated white blood cell count in the blood.

There are many causes of colitis. Infectious colitis may be caused by invasive organisms including *Campylobacter jejuni, Shigella, Salmonella,* and invasive *Escherichia coli.* Amoeba can present in this manner, as can certain infections associated with HIV/AIDS, for example, cytomegalovirus and herpes virus. Noninfectious colitis includes ischemic colitis, drug-induced colitis (as with gold salts or NSAIDs), inflammatory bowel disease (Crohn disease or ulcerative colitis), and radiation injury. *C. difficile* colitis, which will be discussed in the next section, is a relatively new disease that has emerged as a major cause of hospital-acquired infection over the past 40 years largely due to the widespread use of broad-spectrum antibiotics.[109]

C. DIFFICILE

C. difficile colitis is a toxin-induced bacterial disease, which has become increasingly common and progressively more difficult to treat. It is surpassing methicillin-resistant *Staphylococcus aureus* as the leading cause of hospital-acquired infections. Most infections follow antibiotic use, which reduces the normal bacterial flora of the colon and produces a niche for supra-infection by *C. difficile*. As such, *C. difficile* infection only became common following widespread use of broad-spectrum antibiotics in the 1960s.[110] *C. difficile* infection is most commonly associated with, but not limited to, exposure to fluoroquinolones, clindamycin, cephalosporins, and β-lactamase inhibitors.[111] Clinical symptoms of infection range from an asymptomatic carrier state to chronic diarrhea, acute colitis, and life-threatening colitis with sepsis. Severe *C. difficile* colitis is marked by a characteristic appearance of *pseudomembranes*, which consist of inflammatory exudates or yellowish plaques on the colonic mucosa, and is thus referred to as *pseudomembranous colitis.* Milder cases present with inflammation limited to the superficial colonic epithelium; however, in severe cases there can be necrosis of the full thickness of the colonic wall.[112]

Clostridia species have the ability to form spores that can survive extreme environmental conditions and remain viable for years. Spores tend to persist within the hospital environment where they may infect patients receiving antibiotics, causing *C. difficile* to be the most common cause of infectious diarrhea in hospitalized patients.[110]

C. difficile produces clinical disease by secreting various toxins within the colon. Toxins A and B are the most common toxins produced, with >90% of pathogenic strains producing toxin A. These toxins affect the permeability of enterocytes, trigger apoptosis, and stimulate inflammation. Some emerging strains also produce a binary toxin, which is associated with a more severe illness. The bacterium itself is not pathogenic, and some strains of *C. difficile* do not produce toxins and are therefore harmless.[113]

About 3% of healthy adults and 20% of hospitalized patients are asymptomatically colonized with *C. difficile* bacteria.[114] Unlike other similar hospital-infections (e.g., *Staphylococcus aureus*), asymptomatic carriage of *C. difficile* bacteria *actually reduces* the likelihood of developing clinical disease, even following antibiotic exposure. This is probably because people who are asymptomatically colonized have developed antibodies that neutralize the *C. difficile* toxins, or have harmless strains of *C. difficile,* which produce no toxin (yet occupy a niche in the colon preventing infection by toxigenic strains).[115]

Recently, a number of outbreaks have resulted from a new strain of *C. difficile* bacteria, which is resistant to fluoroquinolones (e.g., ciprofloxacin, moxifloxacin.)[116-118] This strain expresses a binary toxin (until now generally not seen in clinical isolates), as well as up-regulates its expression of toxins A and B by about 20-fold. Clinically, this correlates with ominous increases in morbidity and mortality. The continued emergence of *C. difficile* strains with resistance to commonly utilized antibiotics and increased expression of virulence factors suggests that this bacterium will continue to be a serious complication of antibiotic use until a toxin vaccine can be developed. Current treatment consists of metronidazole (oral or IV), vancomycin (oral or rectal), and various probiotics, depending on severity; however, 20–30% of patients who receive therapy will face recurrent *C. difficile* infection.[119] Fidaxomicin, a more recently FDA-approved narrow spectrum macrolide for *C. difficile* infection may serve as a beneficial alternative therapy.[120] A newer approach to treatment of *C. difficile* involves fecal transplantation, where stool is "donated" by a healthy donor and instilled into the GI tract of the infected patient.[121]

Prevention of *C. difficile* infection is largely based on avoidance of antibiotic therapy, unless absolutely necessary, and careful hand washing in hospitals and other institutional settings (including in-home patient care). *C. difficile* spores are somewhat resistant to alcohol-based hand disinfectants, so washing with soap and water is preferred.

Diagnosis

The diagnosis of *C. difficile* is quite challenging. Similar to *H. pylori*, there are a variety of modalities available that vary in sensitivity, specificity, cost, availability, and timeliness. One important difference compared to *H. pylori* is that patients with pseudomembranous colitis may deteriorate rapidly, so making a prompt and accurate diagnosis is important. In some situations that clearly point to a diagnosis of *C. difficile* in an acutely ill patient, it may be reasonable to initiate treatment on an empiric basis before the test results are even available. Diagnosis may also be made during lower endoscopy on encountering the characteristic white or yellow pseudomembranes on the colonic wall.

Until recently, the most commonly used tests for *C. difficile* infection have been ELISA assays for toxin or *C. difficile* antigen within the stool. These tests are available in various commercial kits and have the advantage of being rapid, producing results within hours, and are relatively inexpensive. Tests detect toxin A or both toxins A and B, and have high specificity (typically >95%) but variable sensitivity (60–95%). For this reason, a negative test may be followed by one to two repeat tests to increase the composite sensitivity to the 90% range and exclude infection with more certainty.[114,122] Testing for both toxins has a diagnostic advantage over testing for toxin A because a minority of strains are toxin A-negative and toxin B-positive.[112]

ELISA assays for *C. difficile* common antigen (glutamate dehydrogenase) have improved sensitivity but are less specific because they will detect nontoxigenic species as well as some species of closely related anaerobes. Therefore, a positive assay for *C. difficile* antigen does not prove pseudomembranous colitis and must be followed up with a toxin assay to prove the presence of a pathogenic *C. difficile* strain.[123] The advantage of this assay for *C. difficile* antigen is that the sensitivity is better, such that a single negative assay may be used to exclude the presence of pseudomembranous colitis. The availability, performance, and appropriate use of these assays may vary among hospital laboratories, and inquiries should be made with the laboratory regarding which tests are available and the appropriate strategy for their use.

The "gold standard" test for pseudomembranous colitis has been the detection of toxin A or B in stool samples by demonstrating its cytopathic effect in cell cultures and inhibition of cytopathic effect by specific antiserum.[124,125] Referred to as *cell cytotoxicity assay*, this test has excellent sensitivity (94–100%) and specificity (99%). However, these performance characteristics may be laboratory-dependent.[121,126] Moreover, this test is limited by high cost, a requirement for meticulously maintained tissue culture facilities, and a time delay of one to three days.[125]

C. difficile can also be cultured from stools with selective medium and identified with more traditional microbiologic techniques including colony morphology, fluorescence, odor, gram stain, and signature gas liquid chromatography. Interestingly, this is not the most sensitive test for the organism. In addition, the bacterium is named *difficile* because of *difficulty* in culturing it. Another drawback is that isolated bacteria must then be tested for toxin production to avoid confusing it with nontoxic *C. difficile* strains.[127] Altogether, these factors make bacterial culture and toxin profiling a costly, time-consuming process, and thus they are rarely used. The primary advantage of this approach is that it isolates the organism, allowing genetic tests, which may aid in tracking mutant strains and determining the source of epidemics.[128]

The FDA recently approved three RT-PCR assays for the gene toxin B, which not only provide fast and accurate diagnosis of *C. difficile* but also provide the ability to identify if the pathogen is in the epidemic 027/NAP1/BI strain.[129] This test is rapidly becoming a standard test for initial evaluation of *C. difficile* infections. (**Minicase 12**.)

MINICASE 10

A Case of NASH

Allen K., a 48-year-old corporate executive, presents for his required company physical examination. He had no medical complaints, a negative past medical history, and is not taking any medications. He denies alcohol use.

Physical exam reveals weight 240 lb and height 5'10". His blood pressure is elevated at 154/98. The rest of his physical examination is normal.

Laboratory data include a normal CBC and kidney function, fasting glucose 129 mg/dL, and elevated cholesterol. His LFTs are elevated with ALT 134 IU/L and AST 105 IU/L. His serum bilirubin, albumin, ALP, and INR are all normal.

He is referred for evaluation of his abnormal liver panel, and further testing shows no evidence of viral hepatitis, hemochromatosis, or autoimmune liver disease. The possibility of fatty liver is raised, and a liver biopsy is performed, which shows NASH with early cirrhosis.

QUESTION: What is NASH, and how is it treated?

DISCUSSION: Our society is experiencing a marked increase in the incidence of obesity. Many of these patients develop what is defined as metabolic syndrome, which must have three of the following: abdominal obesity, elevated blood pressure, impaired glucose tolerance, or hyperlipidemia. Obese patients, particularly those with the metabolic syndrome, are at a higher risk of developing NAFLD (nonalcoholic fatty liver disease) or fatty liver. Some patients with fatty liver can progress to NASH and ultimately to cirrhosis and liver failure. Although many drugs have been tried in these cases, ultimately the only accepted treatment is weight loss through diet and occasionally surgery, including laparoscopic gastric bypass or banding. Fatty liver also can be caused by rapid weight loss, hyperalimentation, medications (such as steroids, estrogens, amiodarone), and short bowel syndrome.

MINICASE 11

Diagnosing Pancreatitis

James T., a 55-year-old male, develops a vague but persistent epigastric pain that radiates to his back. He notes that his appetite is "off," and his clothes are getting much looser on him. His pain is not related to eating, activity, or position.

He presents to his primary care physician where it is documented he has lost about 25 pounds in the past year. His physical examination is unremarkable. Laboratory data show a normal CBC, renal function, and LFTs. His amylase and lipase are both elevated with a serum amylase of 189 units/L and a lipase of 390 units/L. Serum calcium and triglycerides are normal.

There are no identifiable precipitating causes noted. The first consideration is that he might have acute or even chronic pancreatitis.

A CT scan is arranged, and it shows a pancreatic mass. He is referred to a surgeon for consideration of surgery, with a presumptive diagnosis of pancreatic carcinoma. James T. and his family, however, get a second opinion. Further workup shows an elevated ANA and a serum IgG4 markedly elevated at 464 mg/dL (normal being up to 140 mg/mL).

The diagnosis of autoimmune pancreatitis is suggested, and James T. is offered a pancreatic biopsy. He elects for a two-week trial of steroids. Prednisone is started at 40 mg/day. Two weeks later, he reports feeling better, and his CT demonstrates a marked reduction in the size of his mass.

QUESTION: How do we diagnose autoimmune pancreatitis?

DISCUSSION: Autoimmune pancreatitis is a new recognized disease where patients can present with findings often indistinguishable from pancreatic cancer or chronic pancreatitis. The diagnosis is suggested if other autoimmune diseases are present, including Sjögren syndrome, autoimmune thyroid diseases, or autoimmune renal diseases. In this patient the presence of a markedly elevated IgG4 level is highly suggestive of this autoimmune disease. Biopsy would likely have been diagnostic. However a rapid response to steroids is virtually diagnostic of this condition. In some cases, the steroids can be tapered down, and some patients will require ongoing immunosuppressive therapy, usually with azathioprine.

The importance of making this diagnosis is in providing appropriate therapy and avoiding what would have been extensive, life-changing surgery. The patient's steroids were tapered over a period of time, and his CT did revert to normal.

MINICASE 12

Antibiotic-Induced Pseudomembranous Colitis

Julia T. presents to her physician after several days of crampy abdominal pain, diarrhea, persistent fever up to 102.5 °F, and chills. On physical examination, she is well hydrated. Her abdomen is soft and nontender. Stools are sent for pathogenic bacterial cultures including *Shigella, Salmonella, Campylobacter,* entero-invasive *E. coli*, and *Yersinia*; meanwhile, Julia T. is given a prescription for diphenoxylate.

Twenty-four hours later, Julia T. presents to the emergency department (ED) doubled over with severe abdominal pain. Her abdomen is distended and tender with diffuse rigidity and guarding. Clinically, she is dehydrated. Her WBC count is elevated at 23,000 cells/mm^3 (3.54–9.06 × 10^3 cells/mm^3), and her BUN is 34 mg/dL. Abdominal x-rays show a dilated colon (toxic megacolon) and an ileus. She then tells the ED physician that, about six weeks earlier, she had taken two or three of her sister's amoxicillin pills because she had thought she was developing a urinary tract infection. Although pseudomembranous colitis is tentatively diagnosed, Julia T. cannot take oral medication because of her ileus. Therefore, IV metronidazole and rectal vancomycin are started. She continues to get sicker, however, and early the next day, most of her colon is removed (the rectum was left intact), and an ileostomy is created.

QUESTION: What is the time course of pseudomembranous colitis? Did the use of diphenoxylate influence the outcome?

DISCUSSION: Pseudomembranous colitis can occur even after only one or two doses of a systemic antibiotic or after topical antibiotic use. Moreover, it can occur up to six weeks after the last dose of antibiotic. A complete history of antibiotic use is critical when dealing with patients with diarrhea.

Diphenoxylate or loperamide use in the face of colitis is associated with an increased risk, although small, of toxic megacolon. In this medical emergency, the colon has no peristalsis; together with the inflammation in the colon wall (colitis), this condition leads to progressive distention. If untreated, perforation and death ensue. The development of a megacolon or ileus in this patient is especially worrisome because the best treatment—oral antibiotics—would be of little benefit. However, IV metronidazole is excreted into the bile in adequate bactericidal levels to eradicate the bacteria. Unfortunately, in the absence of peristalsis, its benefit would be questionable.

SUMMARY

Analysis of liver tests is complex and may be frustrating. Most tests in the LFT panel check for the presence of two broad categories of liver diseases—cholestasis versus hepatocellular injury. Therefore, an abnormal value may raise more questions than it answers. None of the tests are 100% sensitive, and most may be confounded by a variety of factors. How, then, can these tests be used to answer clinical questions with any certainty?

Probably the most important point to bear in mind when interpreting LFTs is that they are but one piece of the puzzle. Correct interpretation relies on interpreting the test within the greater context of the patient, other laboratory data, historical information, and the physical exam.[2,19] For example, mildly elevated bilirubin and ALP in the setting of a critically ill, septic patient is likely cholestasis of sepsis and does not necessarily require extensive evaluation. The same set of laboratory tests (mildly elevated bilirubin and ALP) in an ambulatory patient could be a sign of serious chronic illness such as PBC. However, if this same ambulatory patient had a history of normal LFTs and had recently started taking a medication known to cause cholestasis, then the abnormality would most likely be a side effect of the medication. Thus, the same set of liver tests in three different settings may have widely differing significance.

It is also important to interpret an abnormal value within the context of other laboratory tests, and this is why LFTs are often obtained as a group (i.e., the LFT panel). For example, a mildly elevated AST in the setting of an otherwise normal LFT panel might be of nonhepatic origin (e.g., muscle disease). Alternatively, a mildly elevated AST combined with mildly elevated ALT might raise a concern about a mild hepatocellular process, perhaps chronic viral hepatitis or NASH. Finally, mildly elevated ALT and AST in combination with dramatically elevated ALP and bilirubin would point instead to a cholestatic process.

Therefore, liver tests should always be interpreted with a clear understanding of the clinical context and other laboratory abnormalities. Although the LFT panel will rarely yield an exact diagnosis, it may indicate the type of process (e.g., cholestatic versus hepatocellular) and the severity of the process (e.g., fulminant liver failure versus mild hepatic inflammation). This will lead the practitioner to a group of possibilities that may be further evaluated based on the information at hand, along with other laboratory tests or studies (e.g., radiographs, endoscopic procedures, or tissue biopsies) as needed. The diagnostic yield of these tests also depends on their appropriateness and the thoughtfulness of their selection. Liver studies obtained to answer a specific clinical question (e.g., "Does this patient have liver inflammation due to initiation of statin medications?") are more likely to yield interpretable information than a less guided question ("Is this patient sick?").

Some other aspects of gastroenterology and related laboratory tests are also reviewed in this chapter. Amylase and lipase may reflect pancreatic inflammation; *H. pylori* may be related to ulcer disease; and *C. difficile* is a major cause of hospital-acquired colitis. Although these tests are less convoluted than the LFT panel, it is still paramount to obtain them in a thoughtful manner and interpret the results in the appropriate clinical setting. For example, colonization with *H. pylori* may be of no significance in an asymptomatic patient, whereas it may mandate a course of multiple antibiotics in a patient with recurrent significant gastric ulcer bleeding.

LEARNING POINTS

1. ***Why is the term liver function test a misnomer?***

ANSWER: Often, the term is used to describe a panel of tests including AST, ALT, bilirubin, ALP, and albumin. However, the term is a misnomer because not all of these tests measure liver function. The liver has several functions and different tests reflect these different functions. The table below divides liver tests into rough categories by function and type.

FUNCTION/TYPE	LABORATORY TESTS
Synthetic liver function	Albumin, prealbumin, PT/INR
Excretory function	ALP, 5' nucleotidase, GGT, bilirubin
Hepatocellular injury	AST, ALT
Detoxification	Ammonia

ALP = alkaline phosphatase; ALT = alanine transaminase; AST = aspartate transaminase; GGT = gamma-glutamyl transpeptidase; INR = international normalized ratio; PT = prothrombin time.

2. ***Identify common disorders that cause isolated increased indirect bilirubinemia versus direct bilirubinemia. Explain the pathophysiologic cause of the laboratory abnormality in each case.***

ANSWER: Indirect bilirubin is produced by the breakdown of erythrocytes. Indirect bilirubin is delivered to the liver, where it is converted to direct bilirubin by glucuronyl transferase. Thus, an elevated level of indirect bilirubin may result from increased breakdown of red blood cells (hemolysis) or reduced hepatic conversion of indirect bilirubin to direct bilirubin. Common causes include hemolysis, Gilbert syndrome, or drugs including probenecid or rifampin.

Increased direct bilirubin implies hepatic disease, which interferes with secretion of bilirubin from the hepatocytes or clearance of bile from the liver. Direct bilirubinemia, therefore, is generally classified as a positive cholestatic liver test, although it may also be due to a hepatocellular process. In cholestatic disease, the bilirubin is primarily conjugated, whereas in hepatocellular processes, significant increases in both conjugated and unconjugated bilirubin may result. Cholestasis may be intrahepatic or extrahepatic. Intrahepatic cholestasis may be due to viral hepatitis, alcoholic hepatitis or cirrhosis, pregnancy, severe infection, or PBC. Extrahepatic cholestasis involves obstruction of the large bile ducts outside of the liver, which can be due to strictures, stones, or tumors.

3. ***How is acute pancreatitis diagnosed?***

ANSWER: The clinical presentation of acute pancreatitis generally consists of epigastric pain, often radiating to the back. There can be associated nausea, vomiting, diaphoresis, and fever. The challenge here is that these symptoms are not specific at all. Similar complaints can be seen with biliary disease, ulcers, gastritis, small bowel problems, or compromised blood supply to the gut. At times there can be overlap. Gallstones can migrate down the common bile duct, causing pancreatitis. Ulcers can penetrate the duodenum and invade the pancreas also causing pancreatitis. To establish a diagnosis of pancreatitis one looks for three things. First, the clinical picture should be consistent with this diagnosis. Secondly, a serum lipase or amylase should be over three times normal (realizing that these tests are not specific). Thirdly, it is often of value (especially if the first two criteria are not both present) to have an advanced imaging study, either MRI or CT scan, showing pancreatitis. Generally, two of the three criteria should be present before diagnosing acute pancreatitis.

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QUICKVIEW | Albumin

PARAMETER	DESCRIPTION	COMMENTS
Common reference ranges		
Adults	4–5 g/dL (40–50 g/L)	
Pediatrics	1.9–4.9 g/dL (19–49 g/L)	<1 yr old
	3.4–4.2 g/dL (34–42 g/L)	1–3 yr old
Critical value	<2.5 g/dL (<25 g/L)	In adults
Natural substance?	Yes	Blood protein
Inherent activity?	Increases oncotic pressure of plasma; carrier protein	
Location		
Production	Liver	
Storage	Serum	
Secretion/excretion	Catabolized in liver	Half-life, approximately 20 days
Major causes of…		
High or positive results	Dehydration	
	Anabolic steroids	
Associated signs and symptoms	Limited to underlying disorder	No toxicological activity
Low results	Decreased hepatic synthesis	Seen in liver disease
	Malnutrition or malabsorption	Substrate deficiency
	Protein losses	Via kidney in nephrotic syndrome or via gut in protein-losing enteropathy
	Pregnancy or chronic illness	
Associated signs and symptoms	Edema, pulmonary edema, ascites	At levels <2–2.5 g/dL or <20–25 g/L
After insult, time to…		
Initial depression or positive result	Days	
Lowest values	Weeks	Half-life, approximately 20 days
Normalization	Days	Assumes insult removed and no permanent damage
Drugs often monitored with test	Parenteral nutrition	Goal is increased levels
Causes of spurious results		
Falsely elevated	Ampicillin and heparin	
Falsely lowered	Supine patients, icterus, penicillin	

QUICKVIEW | PT/INR

PARAMETER	DESCRIPTION	COMMENTS
Common reference ranges		
Adults and pediatrics	INR: 0.9–1.1	
	PT: 12.7–15.4 sec	
Critical value	INR: >5	Unless on warfarin
Natural substance?	Yes	
Inherent activity?	Indirect measurement of coagulation factors	
Location		
Production	Coagulation factors produced in liver	
Storage	Carried in bloodstream	
Secretion/excretion	None	
Major causes of…		
Prolonged elevation	Liver failure	Liver unable to produce coagulation factors; prolonged PT or increased INR does not correct with vitamin K
	Malabsorption or malnutrition	Vitamin K aids in activation of coagulation factors and is not absorbed; defect corrects with parenteral vitamin K supplementation
	Warfarin	Corrects with vitamin K supplementation
	Antibiotics	Interfere with vitamin K production by bacteria in the GI tract, or metabolism or activation of clotting factors
Associated signs and symptoms	Increased risk of bleeding and ecchymosis	Easy bruising
Low results	None	
After insult, time to…		
Initial elevation or positive result	6–12 hr	
Peak values	Days to weeks	Depends on etiology
Normalization	4 hr if vitamin K responsive (due to malabsorption, maldigestion, warfarin, etc.) but 2–4 days if due to liver disease and liver disease reverses	
Drugs often monitored with test	Warfarin	
Causes of spurious results	Improper specimen collection	

GI = gastrointestinal; INR = international normalized ratio; PT = prothrombin time.

QUICKVIEW | ALP

PARAMETER	DESCRIPTION	COMMENTS
Common reference ranges		
Adults	Varies with assay	Elevated in pregnancy
Pediatrics	Varies; can be twofold to threefold higher than in adults	Elevated with developing bone
Natural substance?	Yes	Metabolic enzyme (intracellular)
Inherent activity?	Elevation alone causes no symptoms	Intracellular activity only
Location		
Production	Intracellular enzyme	
Storage	Liver, placenta, bone, small intestine, leukocytes	These tissues are rich in ALP
Secretion/excretion	None	
Major causes of…		
High or positive results	Cholestasis	Hepatic; associated with elevation of GGT
	Bone disease	Paget disease, bone tumors, rickets, osteomalacia, healing fracture
	Pregnancy	Placental ALP
	Childhood	Related to bone formation
Associated signs and symptoms	Limited to underlying disorder	Reflects tissue or organ damage
Low results	Vitamin D intoxication	
	Scurvy	
	Hypothyroidism	
Associated signs and symptoms	Limited to underlying disorder	
After insult, time to…		
Initial elevation or positive result	Hours	
Peak values	Days	
Normalization	Days	Assumes insult removed and no ongoing damage
Drugs often monitored with test	None	
Causes of spurious results	Blood drawn after fatty meal and prolonged serum storage	

ALP = alkaline phosphatase; GGT = gamma-glutamyl transpeptidase.

QUICKVIEW | AST

PARAMETER	DESCRIPTION	COMMENTS
Common reference ranges		
Adults	12–38 IU/L (0.2–0.64 μkat/L)	Varies with assay
Newborns/infants	30–100 IU/L (0.5–1.67 μkat/L)	Varies with assay
Critical value	>80 IU/L (1.34 μkat/L)	2 times upper limit of normal
Natural substance?	Yes	Metabolic enzyme
Inherent activity?	None in serum	Intracellular activity only
Location		
Production	Intracellular enzyme	
Storage	Liver, cardiac muscle, kidneys, brain, pancreas, lungs	These tissues are rich in AST
Secretion/excretion	None	
Major causes of…		
High or positive results	Hepatitis	Elevated in any disease with hepatocyte inflammation (liver cells)
	Hemolysis	Elevated in any disease with damage to tissues rich in enzyme
	Muscular diseases	
	Myocardial infarction	
	Renal infarction	
	Pulmonary infarction	
	Necrotic tumors	
Associated signs and symptoms	Varies with underlying disease	Reflects tissue or organ damage
Low results	None	
After insult, time to…		
Initial elevation or positive result	2–6 hr	
Peak values	24–48 hr (without further cell damage)	With extensive liver or cellular damage, levels can go up to thousands
Normalization	24–48 hr	Assumes insult removed and no ongoing damage
Drugs often monitored with test	Isoniazid, HMG-CoA inhibitors, allopurinol, methotrexate, ketoconazole, and valproic acid	Monitoring frequency varies with drug
Causes of spurious results		
Falsely elevated	Heparin, levodopa, methyldopa, tolbutamide, para-aminosalicylic acid, erythromycin, diabetic ketoacidosis	
Falsely lowered	Metronidazole, trifluoperazine, vitamin B_6 deficiency	

AST = aspartate aminotransferase; HMG-CoA = 3-hydroxy-3-methylglutaryl-CoA lyase.

QUICKVIEW | ALT

PARAMETER	DESCRIPTION	COMMENTS
Common reference ranges		
Adults	7–41 IU/L (0.12–0.68 µkat/L)	Varies with assay
Newborns/infants	6–40 IU/L (0.1–0.67 µkat/L)	Decreases to adult values within a few months
Critical value	>60 IU/L (>1 µkat/L)	>2 times normal limit
Natural substance?	Yes	Metabolic enzyme
Inherent activity?	None in serum	Intracellular activity only
Location		
Production	Intracellular enzyme	
Storage	Liver, muscle, heart, kidneys	These tissues are rich in ALT
Secretion/excretion		Normally contained intracellularly, but with cell damage, serum concentrations increase
Major causes of…		
High or positive results	Hepatitis	Elevated in any disease with hepatocyte inflammation (liver cells)
	Hemolysis	Elevated in any disease with damage to tissues rich in enzymes
	Muscular diseases	
	Myocardial infarction	
	Renal infarction	
Associated signs and symptoms	Varies with underlying disease	Reflects tissue or organ damage
Low results	Patients deficient in vitamin B_6	
Associated signs and symptoms	None	
After insult, time to…		
Initial elevation or positive result	2–6 hr	
Peak values	24–48 hr (without further cell damage)	With extensive liver or cellular damage, levels can go up to thousands
Normalization	24–48 hr	Assumes insult removed and no ongoing damage
Drugs often monitored with test	Isoniazid and cholesterol-lowering agents (e.g., HMG-CoA inhibitors, allopurinol, ketoconazole, valproic acid, and methotrexate)	Monitoring frequency varies with drug
Causes of spurious results	Heparin (false elevation)	

ALT = alanine aminotransferase; HMG-CoA = 3-hydroxy-3-methylglutaryl-CoA lyase.

QUICKVIEW | Bilirubin

PARAMETER	DESCRIPTION	COMMENTS
Common reference ranges		
Adults	0.3–1.3 mg/dL (5.1–22.2 μmol/L)	Varies slightly with assay
Pediatrics	2–4 mg/dL (34.2–68.4 μmol/L)	24-hr infant
	5–6 mg/dL (85.5–102.6 μmol/L)	48-hr infant
	0.3–1.3 mg/dL (5.1–22.2 μmol/L)	>1 mo old
Critical value	>4 mg/dL (>68.4 μmol/L)	In adults
Natural substance?	Yes	Byproduct of Hgb metabolism
Inherent activity?	Yes	CNS irritant or toxin in high levels in newborn (not adult)
Location		
Production	Liver	
Storage	Gallbladder	Excreted into bile
Secretion/excretion	Stool and urine	Bilirubin and urobilinogen
Major causes of…		
High or positive results	Liver disease, both hepatocellular and cholestatic	
	Hemolysis	
	Metabolic abnormalities (e.g., Gilbert syndrome)	
Associated signs and symptoms	Jaundice	
Low results	No important causes	
After insult, time to…		
Initial elevation or positive result	Hours	
Peak values	3–5 days	Assumes insult not removed
Normalization	Days	Assumes insult removed and no evolving damage
Drugs often monitored with test	None	
Causes of spurious results	Fasting, levodopa, phenelzine, methyldopa, ascorbic acid (false elevation)	

CNS = central nervous system; Hgb = hemoglobin.

QUICKVIEW | Ammonia

PARAMETER	DESCRIPTION	COMMENTS
Common reference ranges		
Adults and pediatrics	19–60 mcg/dL (13.6–42.8 μmol/L)	Varies with assay
Newborns	<100 mcg/dL (71.4 μmol/L)	Varies with assay
Critical value	Varies, generally 1.5 upper limit of normal	
Natural substance?	Yes	Product of bacterial metabolism of protein (in the gut)
Inherent activity?	Probably	Progressive deterioration in neurologic function
Location		
Production	In gut (by bacteria)	
Storage	None	
Secretion/excretion	Liver metabolizes to urea	Urea cycle; diminished in cirrhosis
Major causes of…		
High or positive results	Liver failure	
	Reye syndrome	
	Metabolic abnormalities (urea cycle)	
Associated signs and symptoms	Hepatic encephalopathy	
Low results	No important causes	
After insult, time to…		
Initial elevation or positive result	Hours	
Peak values	No peak value; rises progressively	
Normalization	Days	After appropriate therapy or resolution of underlying liver disease
Drugs often monitored with test	Valproic acid	
Causes of spurious results	Sensitive test (discussed in text)	

16

HEMATOLOGY: RED AND WHITE BLOOD CELL TESTS

Paul R. Hutson

OBJECTIVES

After completing this chapter, the reader should be able to

- Describe the physiology of blood cell development and bone marrow function
- Discuss the interpretation and alterations of hemoglobin, hematocrit, and various red blood cell indices in the evaluation of macrocytic, microcytic, and normochromic/normocytic anemias
- Describe the significance of abnormal erythrocyte morphology, including sickling, anisocytosis, and nucleated erythrocytes
- Name the different types of leukocytes and describe their primary functions
- Calculate the absolute number of various types of leukocytes from the white blood cell count and differential
- Interpret alterations in the white blood cell count, differential, and CD_4 lymphocyte count in acute bacterial infections, parasitic infections, and human immunodeficiency virus infection
- Identify potential causes of neutrophilia
- Identify how leukocyte phenotypes are used for antitumor drug selection

This chapter reviews the basic functions and expected laboratory values of erythrocytes and leukocytes. It also discusses, in an introductory manner, selected disorders of these two cellular components of blood. It must be remembered that the ability of laboratory medicine to discriminate between leukocytes is increasing, and many methods considered investigational in this edition may become a routine component of blood examination in the future.

PHYSIOLOGY OF BLOOD CELLS AND BONE MARROW

The cellular components of blood are derived from pluripotential stem cells located in the bone marrow that can differentiate into red blood cells (RBCs), white blood cells (WBCs), and platelets (**Figure 16-1**). *Bone marrow* is a highly structured and metabolically active organ, which normally produces 2.5 billion RBCs, 1 billion granulocytes, and 2.5 billion platelets/kg of body weight daily.[1] Production can vary greatly from nearly 0 to 5 to 10 times normal. Usually, however, levels of circulating cells remain in a relatively narrow range (**Table 16-1**).[2]

In the fetus and children, blood cell formation or hematopoiesis occurs in the marrow of virtually all bones, as well as in liver, spleen and other visceral organs. With maturation, the task of hematopoiesis ceases in the liver and shifts to flat bones of the axial skeleton such as the cranium, ribs, pelvis, and vertebrae. The long bones, such as the femur and humerus, do not produce a large amount of blood cells in adulthood because the marrow is gradually replaced by fatty tissue.

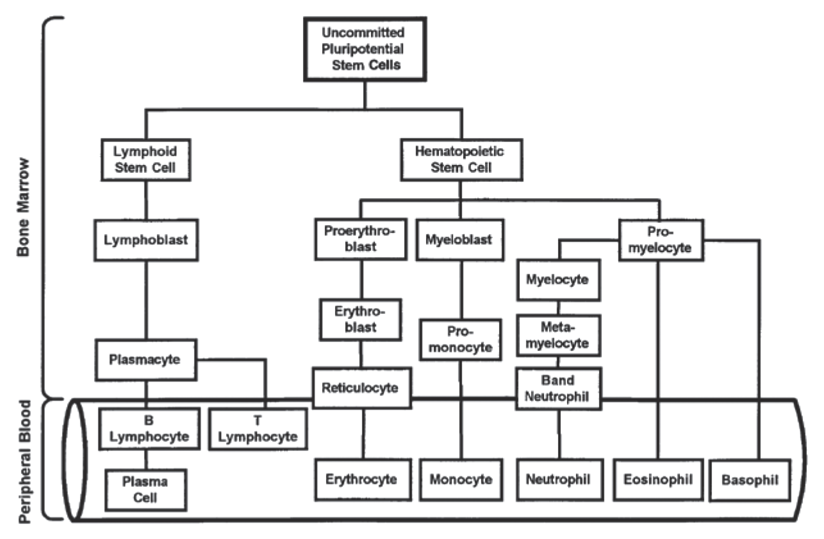
Uncommitted Pluripotential Stem Cells
Lymphoid Stem Cell
Hematopoietic Stem Cell
Bone Marrow
Lymphoblast
Proerythro-blast
Myeloblast
Pro-myelocyte
Myelocyte
Erythro-blast
Pro-monocyte
Meta-myelocyte
Plasmacyte
Reticulocyte
Band Neutrophil
Peripheral Blood
B Lymphocyte
T Lymphocyte
Plasma Cell
Erythrocyte
Monocyte
Neutrophil
Eosinophil
Basophil

FIGURE 16-1. Schematic diagram of hematopoiesis.

Note: The author wishes to thank Irene Chung for her skilled assistance in the revision of this chapter.

TABLE 16-1. Reference Ranges and Interpretative Comments for Common Hematological Tests (Typical CBC)[a]

TEST NAME	RANGE[a] REFERENCE	SI UNITS	COMMENTS
RBC	Males: 4.5–5.9 × 10^6 cells/µl Females: 4.1–5.1 × 10^6 cells/µl	4.5–5.9 × 10^{12} cells/L 4.1–5.1 × 10^{12} cells/L	
Hgb	Males: 14–17.5 g/dL Females: 12.3–15.3 g/dL	140–175 g/L or 8.68–10.85 mmol/L 123–153 g/L or 7.63–9.49 mmol/L	Amount of Hgb in given volume of whole blood; indication of oxygen-transport capacity of blood; elevated in hyperlipidemia
Hct	Males: 42–50% Females: 36–45%	0.42–0.5 0.36–0.45	Percentage volume of blood comprised of erythrocytes; usually approximately three times Hgb
RBC indices MCV	80–96 fL/cell	80–96 fL/cell	Hct/RBC: average size of RBCs in a specimen; increased in vitamin B_{12} and folate deficiency, cold agglutinins, reticulocytosis, hyperglycemia, and leukemic cells; decreased in iron deficiency
MCH	27–33 pg/cell	27–33 pg/cell	Hgb/RBC: average amount of hemoglobin in RBCs in a specimen; increased in vitamin B_{12} and folate deficiency; decreased in iron deficiency
MCHC	33.4–35.5 g/dL	334–355 g/L	Hgb/Hct: average concentration of Hgb in RBCs in a specimen; increased in hyperlipidemia and cold agglutinins; decreased in iron deficiency
Reticulocyte count	0.5–2.5% of RBCs	0.005–0.025	Immature RBCs; increased in acute blood loss and hemolysis; decreased in untreated iron, vitamin B_{12}, and folate deficiency
RDW	11.5–14.5%	0.115–0.145	Measure of variation in RBC volumes (anisocytosis): the larger the width percent, the greater the variation in size of RBCs; increased in early iron deficiency anemia and mixed anemias
WBC count	4.4–11.3 × 10^3 cells/µL	4.4–11.3 × 10^9 cells/L	Elevated by neutrophil demargination with exercise, glucocorticoids, epinephrine; decreased with cold agglutinins
Platelet count	150,000–450,000 cells/µL	150–450 × 10^9 cells/L	Elevated in presence of RBC fragments and microcytic erythrocytes; decreased in presence of large numbers of giant platelets and platelet clumps
MPV	6.8–10 fL	6.8–10 fL	

Hct = hematocrit; Hgb = hemoglobin; RBC/s = red blood cell/s; RDW = RBC distribution width; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; MPV = mean platelet volume; SI = International System of Units; WBC = white blood cell.

[a] Modified from references 8 and 9.

Radiation directed to large portions of hematopoietic bones can lead to deficient hematopoiesis in patients treated for cancerous lesions such as bony metastases. Similarly, preparation for a bone marrow transplant will often include total body irradiation to destroy the hematopoietic cells of the recipient in the bone, spleen, and other sites so that the grafted cells will not be destroyed by residual host defenses.

Although the majority of hematopoiesis occurs in the marrow, modern methods of identifying cellular characteristics have demonstrated that pluripotential cells—identified by a cellular expression of the surface marker CD34—also normally circulate in the blood.[3,4]

Although the majority of this chapter discusses laboratory analysis of blood obtained from the vein (peripheral venipuncture), an analysis of the bone marrow itself may be needed to diagnose or monitor various disease states, most commonly the leukemias discussed later in the chapter. Bone marrow specimens are usually obtained from the posterior iliac crest of the pelvis or, less commonly because of increased risk, from the sternum. Bone marrow sampling can involve an aspirate, a core biopsy, or both. After penetrating the bone cortex with the bone marrow needle and entering the medullary cavity inside the bone containing the marrow, a heparinized syringe is attached to the needle and 1 to 2 mL of bone marrow is aspirated. The contents of the syringe are smeared on a series of slides that are stained and examined microscopically. If special studies such as flow cytometry or cytogenetics are requested, additional heparinized syringes are aspirated and submitted to the laboratory. The bone marrow biopsy is obtained with the same needle by advancing it further past the site of aspiration through the bone marrow to cut a sample of the bone marrow matrix for removal and examination. The biopsy provides the advantage of examining the structure of the marrow stroma, as well as the spatial relationship of the various hematopoietic cells.[5]

Blood pluripotential (stem) cells become increasingly differentiated in the bone marrow until they are committed to develop further into erythrocytes, platelets, or various leukocytes (Figure 16-1). Many regulatory proteins including colony-stimulating factors are involved in the differentiation and proliferation phases of hematopoiesis, but their functions and interrelationships are not yet fully understood. In addition to the colony-stimulating factors mentioned above, proteins that stimulate hematopoiesis include erythropoietin, thrombopoietin, and various interleukins. Inhibitors of hematopoiesis are not as well defined but include interferons and lymphotoxins. In considering the response of neutrophils or erythrocytes to exogenously administered hematopoietic stimulants (e.g., filgrastim or erythropoietin), it is important to recall that normal physiologic hematopoietic regulation is more complex than the effect of one therapeutic protein would suggest. WBC formation involves local production of a combination of signaling proteins by cells of the hematopoietic microenvironment (e.g., macrophages, T lymphocytes, osteoblasts, fibroblasts, and endothelial cells). Leukocyte-stimulating proteins, such as G-CSF (granulocyte-colony stimulating factor) and GM-CSF (granulocyte-macrophage colony-stimulating factor), are normally directed toward adjacent or closely approximated differentiating hematopoietic cells.[4] In contrast, renal synthesis of the hormone erythropoietin is increased when oxygen tension in one or both kidneys decreases. Once it is released into the systemic circulation, erythropoietin stimulates erythrocyte precursors in the blood-forming areas of bone marrow.

Committed blood precursor cells undergo further differentiation in the bone marrow until they develop into mature cells. These developmental stages can be identified by differing morphological or immunochemical staining characteristics. These same imaging techniques are used to identify the developmental phenotype of the cancerous WBCs of leukemia and lymphoma. Generally, only mature cellular forms are found in the circulating blood, and it is from this blood that clinical specimens are usually taken. As discussed below, the presence of immature forms of WBCs or RBCs in the blood typically indicates the presence of a pathologic process.

COMPLETE BLOOD COUNT

The *complete blood count* (CBC) is a frequently ordered laboratory test. It supplies useful information regarding the concentration of the different cellular and noncellular elements of blood and applies to multiple disorders. CBC is a misnomer because concentrations of cells/μL, not counts, are measured and reported. Functionally, the CBC can be thought of as a complete blood analysis because a series of tests are performed. Moreover, information besides concentrations is reported.

Most clinical laboratories utilize an automated method to determine the CBC. Results are usually accurate, reproducible, and rapidly obtained. Numerous measured and calculated values are included in a CBC (Table 16-1). These results traditionally include the following:

- Erythrocyte (RBC) count
- Leukocyte (WBC) count
- Hemoglobin (Hgb)
- Hematocrit (Hct)
- RBC indices: mean corpuscular volume (MCV), mean Hgb content (MHC), mean corpuscular Hgb concentration (MCHC), and RBC distribution width (RDW); also commonly referred to as *Wintrobe indices*
- Platelet count and mean platelet volume
- Reticulocyte count
- Erythrocyte sedimentation rate (ESR)

When a "CBC with differential" is ordered, the various types of WBCs are also analyzed (see White Blood Cell Count and Differential section). The reliability of the results can be doubtful if (1) the integrity of the specimen is questionable (inappropriate handling or storage), or (2) the specimen contains substances that interfere with the automated analysis. Grossly erroneous results are usually flagged for verification by another method. Manual microscopic review of the blood smear is used to resolve unusual automated results.[1]

Red Blood Cell Count

Normal adult range: males 4.5–5.9 × 10^6 cells/μL
(4.5–5.9 × 10^{12} cells/L); females 4.1–5.1 × 10^6 cells/μL
(4.1–5.1 × 10^{12} cells/L)

The *red blood cell (RBC) count* is the number of red corpuscles in a given volume of blood. The international unit for reporting blood cells is for a one liter volume, but it is still common to see values reported in cells/microliter (μL), or less commonly in cells/cubic millimeter (mm^3). After puberty, females have slightly lower counts (and Hgb) than men, partly because of their menstrual blood loss and because of higher concentrations of androgen (an erythropoietic stimulant) in men. The RBC count in all anemias is by definition below the normal range. This decrease causes a proportionate decrease in Hct and Hgb. The reticulocyte is the cell form that precedes the mature RBC or erythrocyte. During the entire maturation process, Hgb is produced, gradually filling the cytoplasm. The reticulocyte does not contain a nucleus but possesses nucleic acids that can be considered remnants of the nucleus or endoplasmic reticulum. The mature erythrocyte contains neither an organized nucleus nor nucleic acids. Reticulocytes persist in the circulation for one to two days before maturing into erythrocytes.[2,6,7]

Mature erythrocytes have a median lifespan of 120 days under normal conditions. They are removed from the circulation by macrophages in the liver, spleen, bone marrow, and other reticuloendothelial organs. The erythrocytes are tested for flexibility, size, and integrity in these organs as the cells pass through areas of osmotic, pH, or hypoxic stress.[6]

Variability in the size of RBCs is termed *anisocytosis*, and variation in the normal biconcave disc shape is termed *poikilocytosis*. Such abnormalities are seen with iron deficiency or periods of increased erythrocyte production and RBC damage.[7]

White Blood Cell Count

Normal range: 4.4–11.3 × 10^3 cells/μL
(4.4–11.3 × 10^9 cells/L)

The *white blood cell (WBC) count* is an actual count of the number of leukocytes in a given volume of blood. Unlike RBCs, leukocytes have a nucleus and normally represent five different mature cell types. The various percentages of the five mature and WBC types comprise the WBC differential, which is discussed later in this chapter.

Hemoglobin

Normal range: males 14–17.5 g/dL (140–175 g/L);
females 12.3–15.3 g/dL (123–153 g/L)

The *hemoglobin* (Hgb) value is the amount of this metalloporphyrin-protein contained in a given volume (100 mL or 1 L) of whole blood. The Hgb concentration provides a direct indication of the oxygen-transport capacity of the blood. As the major content of the RBCs, Hgb is proportionately low in patients with anemia.

Hematocrit

Normal range: males 42–50% (0.42–0.5);
females 36–45% (0.36–0.45)

The *hematocrit* (Hct) is the percentage volume of blood that is composed of erythrocytes. It is also known as the packed cell volume. To manually perform the Hct test, a blood-filled capillary tube is centrifuged to settle the erythrocytes. Then, the percentage volume of the tube that is composed of erythrocytes is calculated.[5] The Hct is usually about three times the value of the Hgb, but disproportion can occur when cells are substantially abnormal in size or shape. Like Hgb, Hct is low in patients with anemia.

Red Blood Cell Indices

Because the following laboratory tests specifically assess RBC characteristics, they are called *RBC indices*. These indices are useful in the evaluation of anemias, polycythemia, and nutritional disorders. Essentially, they assess the size and Hgb content of the RBC. The MCV is measured directly, while the MCHC and MCH are calculated from the Hgb, MCV, and RBC count using predetermined formulas. Because of its dependence on cell size, MCH is not used clinically, but rather the MCHC is used to assess RBCs for their Hgb concentration and color.

Mean Corpuscular Volume

Normal range: 80–96 fL/cell (80–96 μm^3/cell)

The *mean corpuscular volume* (MCV) is an estimate of the average volume of RBCs and is the most clinically useful of the RBC indices. It can be calculated by dividing the Hct by the RBC count, but it is now determined by averaging the directly measured size of thousands of RBCs with modern hemocytometry instruments.

Abnormally large cells have an increased MCV and are called *macrocytic*. Vitamin B_{12} and folate deficiency cause the formation of macrocytic erythrocytes, which corresponds to a true increase in MCV. In contrast, a false increase in MCV may be observed when a patient has reticulocytosis, an increase in the number of reticulocytes in the peripheral blood, because reticulocytes are larger than mature erythrocytes.[5] The MCV may also be falsely increased in hyperglycemia due to osmotic expansion of the erythrocyte. When erythrocytes are mixed with diluting fluid to perform the test, the cells swell because the diluent is relatively hypotonic compared to the patient's hyperglycemic blood. Abnormally small cells (with a decreased MCV) are called *microcytic*. A decrease in the MCV implies some abnormality in Hgb synthesis. The most common cause of microcytic erythrocytes (microcytosis) is iron deficiency.[10]

Mean Corpuscular Hemoglobin

Normal range: 27–31 pg/cell

The *mean corpuscular hemoglobin* (MCH) is a measure of the oxygen-carrying capacity (i.e., Hgb) of each cell. It is calculated as the quotient of Hgb/RBC. The presence of Hgb adds color to the erythrocyte and also picks up the dyes of RBC stains for microscopic viewing. Cells that have decreased amounts of Hgb are referred to as being *hypochromic*, such as in iron deficiency.

Mean Corpuscular Hemoglobin Concentration

Normal range: 33.4–35.5 g/dL (334–355 g/L)

The *mean corpuscular hemoglobin concentration* (MCHC) is the Hgb divided by the Hct. As mentioned previously, this calculation is usually around 33 g/dL (330 g/L) because the Hct is usually three times the Hgb. Some laboratories do not report the MCH, as the MCHC reports the Hgb per volume of blood rather than per erythrocyte and, therefore, provides a more direct index of the oxygen carrying capacity of the blood. Iron deficiency is the only anemia in which the MCHC is routinely low, although it can also be decreased in other disorders of Hgb synthesis.[8,9] In this case, RBCs are described as hypochromic (pale). MCHC can be falsely elevated in hyperlipidemia. The Wintrobe indices are averages for the patient's blood, and normal values may be reported by automated methods even in the presence of a mixed (normal + abnormal) erythrocyte population.

Red Blood Cell Distribution Width

Normal range: 11.5–14.5% (0.115–0.145)

The *red blood cell distribution width* (RDW) is an indication of the variation in RBC size, termed *anisocytosis*.[8] The RDW is reported as the coefficient of variation of the MCV (standard deviation/mean value). This value is used primarily with other tests to differentiate iron deficiency anemia from thalassemias. The RDW increases in macrocytic anemias and in early iron deficiency, often before other tests show signs of this kind of anemia. However, it is not specific for iron deficiency anemia. Mild forms of thalassemia often are microcytic but have a normal or only slightly elevated RDW.

Platelet Count and Mean Platelet Volume

Normal range: 150,000–450,000 cells/μL (150–450 × 10^9 cells/L)

The *platelet count*, often included routinely in the CBC with differential, and *mean platelet volume* are discussed with other coagulation tests in Chapter 17.

Reticulocyte Count

Normal range: 0.5–2.5% of RBCs (0.005–0.025)

In anemia, the *reticulocyte count* or *reticulocyte index* (RI) reflects not only the level of bone marrow production but also a decline in the total number of mature erythrocytes that normally dilute the reticulocytes. Therefore, the reticulocyte count would double in a person whose bone marrow production is unchanged, but whose Hct has fallen from 46% to 23%. The RI was used with hand-counting methods to correct for a low Hct but is not necessary with modern, automated cell counters.[11]

In persons with anemia secondary to acute blood loss or hemolysis, even the corrected reticulocyte count is increased.[6] This increase reflects an attempt by the bone marrow to compensate for the lack of circulating erythrocytes. Because RBC production is increased above basal activity, more reticulocytes escape into the circulation earlier than normal. In contrast, persons with untreated anemia secondary to iron, folate, or vitamin B_{12} deficiency are unable to increase their reticulocyte count appropriate to the degree of their anemia. Similarly, appropriate treatment of an anemia should be accompanied by an increase in the reticulocyte count.

The reticulocyte count can be useful in identifying drug-induced bone marrow suppression in which the percentage of circulating reticulocytes should be close to zero. It can also be used to monitor an anemic patient's response to vitamin or iron therapy. In such patients, supplementation of the lacking factor causes rapid (five to seven days) elevation of the reticulocyte count.

ERYTHROCYTE SEDIMENTATION RATE

Normal range: males 1–15 mm/hr;
females 1–20 mm/hr (increases with age)

Numerous physiological and disease states are associated with the rate at which erythrocytes settle from blood, termed the *erythrocyte sedimentation rate* (ESR*)*. Erythrocytes normally settle slowly in plasma but settle rapidly when they aggregate because of electrostatic forces. Each cell normally has a net negative charge and repels other erythrocytes because like charges repel each other. Many plasma proteins are positively charged and are attracted to the surface charge of one or more erythrocytes, thereby promoting erythrocyte aggregation.[12] Nonmicrocytic anemia, pregnancy, multiple myeloma, and various inflammatory diseases (including infections) can increase the ESR. Sickle cell disease, high doses of corticosteroids, liver disease, microcytosis, carcinomas, and congestive heart failure can decrease the ESR.[8]

Although the ESR may be used to confirm a diagnosis supported by other tests, it is rarely used alone for a specific diagnosis. Rather, the ESR is useful for monitoring the activity of inflammatory conditions (e.g., temporal arteritis, polymyalgia rheumatica, rheumatoid arthritis, and osteomyelitis).[12] The ESR is often higher when the disease is active due to increased amounts of circulating proteins, termed *acute phase reactants* (e.g., fibrinogen), and falls when the intensity of the disease decreases.

The ESR is usually measured using either the Wintrobe or the Westergren method. Anticoagulated blood is diluted and placed in a perfectly vertical glass tube of standard size. After one hour, the distance from the plasma meniscus down to the top of the erythrocyte column is recorded as the ESR in millimeters per hour.[12] A corrected sedimentation rate, called the *zeta-sedimentation rate* or *ratio*, has been developed to eliminate the effect of anemia on the ESR.[13] This value is called the *zetacrit,* and its normal range is 40–52%.[8] Elevations above the normal range are interpreted in the same manner as an elevated ESR by traditional methods.

LABORATORY ASSESSMENT OF ANEMIA

The functions of the erythrocyte are to transport and protect Hgb, the molecule used for oxygen and carbon dioxide transport. *Anemia* can be caused as a decrease in either the Hct or the Hgb concentration below the normal range for age and gender. Anemia is not a disease in itself but one manifestation of an underlying disease process. Appropriate treatment of the anemic patient depends on the exact cause of the condition. Signs and symptoms of anemia depend on its severity and the rapidity with which it has developed. Severe, acute blood loss results in more dramatic symptoms than an anemia that took months to develop because with chronic loss some compensatory adaptation may occur. Patients with mild anemia are often asymptomatic (i.e., absence of pallor, weakness, and fatigue), but severely symptomatic patients manifest shortness of breath, tachycardia, and palpitations even at rest. This contrast should be kept in mind when interpreting test results.

Anemia can be caused by decreased production and increased destruction of erythrocytes as well as acute blood loss. The first two situations can often be differentiated by the reticulocyte count, which is decreased in the former and increased in the latter. Use of erythrocyte morphology is one common method to characterize the possible etiology of anemia. This method is useful because different causes of anemia lead to different erythrocyte morphology. **Figure 16-2** outlines this approach. Only a few common causes of anemia are included, but others can be fit into this outline. Other laboratory tests that are useful in differentiating the anemias are described below. Usual laboratory findings are also included in each section (**Table 16-2**).

Macrocytic Anemia

Macrocytic anemia is a lowered Hgb value characterized by abnormally enlarged erythrocytes. The two most common causes are vitamin B_{12} and folic acid deficiencies. Drugs that cause macrocytic anemia mainly interfere with proper utilization, absorption, and metabolism of these vitamins (**Table 16-3**). **(Minicase 1.)**

Vitamin B_{12} Deficiency

Vitamin B_{12} is also known as *cobalamin*. The normal daily requirement of vitamin B_{12} is 2–5 mcg.[14] It is stored primarily in the liver, which contains approximately 1 mcg of vitamin/g of liver tissue. Overall, the body has B_{12} stores of approximately 2–5 mg. Therefore, if vitamin B_{12} absorption suddenly ceased in a patient with normal liver stores, several years would pass before any abnormalities occurred due to vitamin deficiency.

The absorption of vitamin B_{12} is complex, and the mechanisms responsible are still being defined. Cobalamin

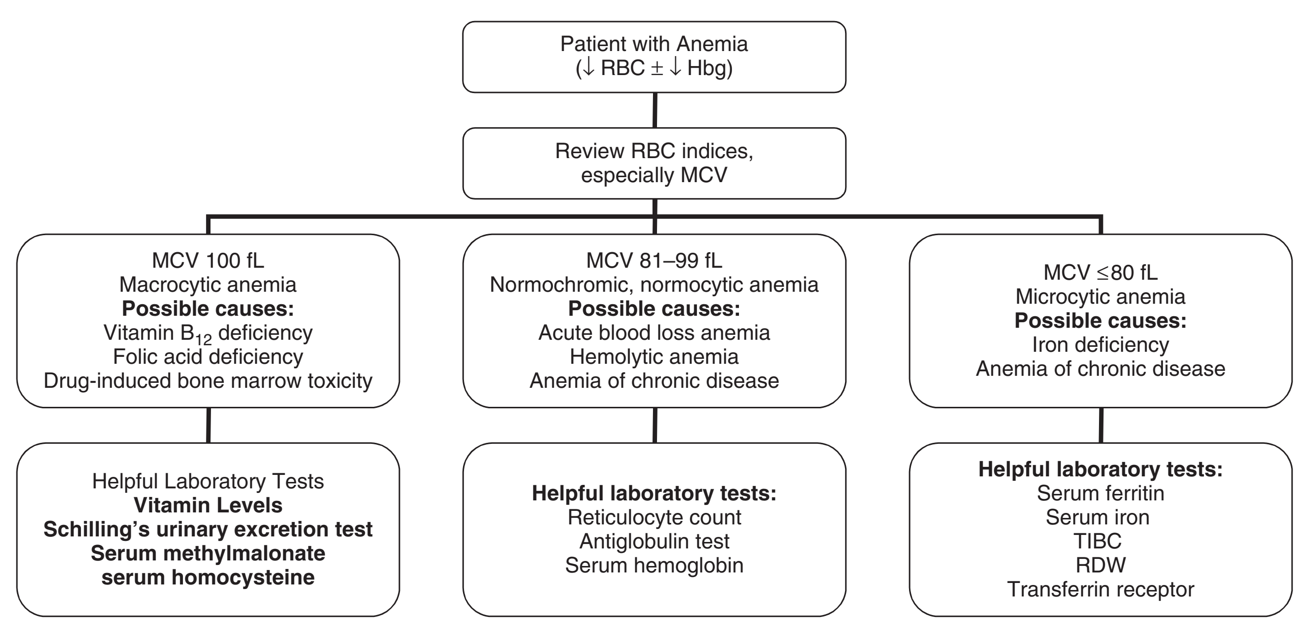
Patient with Anemia
(↓ RBC ± ↓ Hbg)
Review RBC indices, especially MCV
MCV 100 fL
Macrocytic anemia
Possible causes:
Vitamin B_{12} deficiency
Folic acid deficiency
Drug-induced bone marrow toxicity
MCV 81–99 fL
Normochromic, normocytic anemia
Possible causes:
Acute blood loss anemia
Hemolytic anemia
Anemia of chronic disease
MCV ≤80 fL
Microcytic anemia
Possible causes:
Iron deficiency
Anemia of chronic disease
Helpful Laboratory Tests
Vitamin Levels
Schilling's urinary excretion test
Serum methylmalonate
serum homocysteine
Helpful laboratory tests:
Reticulocyte count
Antiglobulin test
Serum hemoglobin
Helpful laboratory tests:
Serum ferritin
Serum iron
TIBC
RDW
Transferrin receptor

FIGURE 16-2. Use of erythrocyte morphology in differential diagnosis of anemia.

TABLE 16-2. Qualitative Laboratory Findings for Various Types of Anemia[a,b]

	VITAMIN B_{12} DEFICIENCY	FOLATE DEFICIENCY	IRON DEFICIENCY	ACUTE BLOOD LOSS	HEMOLYTIC ANEMIA	ANEMIA OF CHRONIC DISEASE
RBC	↓	↓	↓	↓	↓	↓
Hgb	↓	↓	↓	↓	↓	↓
Hct	↓	↓	↓	↓	↓	↓
MCV	↑	↑	↓	↔	↔	↔↓
MCH	↑	↑	↓	↔	↔	↔↓
MCHC	↔	↔	↔	↔		↔↓
RDW	↑	↑	↑	↔	↔	↔
Reticulocyte count	↓	↓	↑	↑	↑	↔↓
Serum vitamin B_{12}	↓	↔				
Serum folate	↔	↓				
Serum methylmalonate	↑	↔				
Serum homocysteine	↑	↑				
Ferritin			↓			↔
Serum iron			↓			↓
TIBC			↑			↓
Serum haptoglobin					↓	
Plasma hemoglobin					↑	
Autoantibodies					+	

Hct = hematocrit; Hgb = hemoglobin; RBC = red blood cell; RDW = RBC distribution width; MCH = mean corpuscular hemoglobin; MCV = mean corpuscular volume; TIBC = total iron-binding capacity.

[a] Some tests with no change (↔) are left empty for clarity.

[b] Autoantibodies positive for antibody-mediated immune hemolysis.

TABLE 16-3. Examples of Causes of Drug-Induced Macrocytic Anemia[16]

Altered folate absorption	
Alcohol	Aminosalicylic acid
Multiple antimalarials	Erythromycin
Estrogen	Oral contraceptives
Various penicillins	Phenytoin
Vitamin B_{12} malabsorption	
Colchicine	Isoniazid
Metformin	Neomycin
Para-aminosalicylic acid	Proton pump inhibitors
Altered purine metabolism	
Allopurinol	Azathioprine
Fludarabine	Cladribine
Mercaptopurine	Methotrexate
Mycophenolate mofetil	Pentostatin
Thioguanine	
Altered pyrimidine synthesis	
Capecitabine	Cytosine arabinoside
Fluorouracil	Gadolinium
Gemcitabine	Hydroxyurea
Leflunomide	Methotrexate
Mercaptopurine	Nitrous oxide
Teriflunomide	Trimethoprim
Vitamin B_{12} inactivation	
Nitrous oxide	

Source: Reprinted with permission from Massachusetts Medical Society. Hesdorffer CS, Longo DL. Drug-induced megaloblastic anemia. N Engl J Med. 2015;373(17):1649–1658. Copyright ©2015; Massachusetts Medical Society.

(vitamin B_{12}) is ingested in meats, eggs, and dairy products. Strict vegans, who avoid all such foods in their diet, may over time develop vitamin B_{12} deficiency if supplements are not ingested. Some forms of B_{12}, such as those made by the blue-green algae *Spirulina*, are active vitamins in bacterial assays but are not active vitamins for humans. Other cobamides structurally related to cobalamin are found in plasma after ingesting other animal and plant-based foods. Only the cobamide with an attached 5,6-dimethylbenzimidazole group is correctly termed *cobalamin* and is active in humans.[14]

Dietary B_{12} or cobalamin is usually bound nonspecifically to proteins, and gastric acid and pepsin are required to hydrolyze the vitamin from the protein. Aging patients with decreasing stomach acid production may be less able to free vitamin B_{12} from meat protein. Freed B_{12} is bound with very high affinity to protein R, which is a large protein secreted in saliva. The cobalamin-protein R complex moves to the duodenum where proteases denature protein R and allow the freed vitamin to bind to intrinsic factor, which is secreted in the stomach and is resistant to the intestinal proteases. Patients may develop autoantibodies to intrinsic factor and, thereby, develop vitamin B_{12} deficiency. The cobalamin-intrinsic factor complex binds to cubulin at the ileal epithelium. The cobalamin translocates, dissociates, and then enters the circulation on the basolateral side of the cell bound to transcobalamin, which is largely

MINICASE 1

Anemia with Increased MCV

Anna B., a 45-year-old female alcoholic, is admitted to the hospital because of pneumonia. Her physical exam reveals an emaciated patient with ascites, dyspnea, fever, cough, and weakness. No cyanosis, jaundice, or peripheral edema is evident. Her peripheral neurologic exam is within normal limits as is her serum electrolytes, urea nitrogen, creatinine, and glucose. The following CBC is obtained:

TEST NAME	RESULT	REFERENCE RANGE
RBC	3×10^6 cells/µL	$4.1–5.1 \times 10^6$ cells/µL for females
WBC	4.6×10^3 cells/µL	$4.4–11.3 \times 10^3$ cells/µL
Hgb	10.3 g/dL	12.3–15.3 g/dL for females
Hct	30.9%	36–45% for females
MCV	110.8 fL/cell	80–96 fL/cell
RDW	15.4%	11.5–14.5%
Platelet	174,000 cells/µL	150,000–450,000 cells/count µL
Neutrophils	68%	45–73%
Bands	6%	3–5%
Monocytes	11%	2–8%
Eosinophils	2%	0–4%
Basophils	2%	0–1%
Lymphocytes	11%	20–40%

QUESTION: What abnormalities are present? What is the likely cause?

DISCUSSION: The patient has anemia, evidenced by the low RBC, Hgb, and Hct. The increased MCV identifies this as a macrocytic anemia. The RDW is elevated, indicating variability in the size of the erythrocytes. These findings are typical of folic acid deficiency, a common finding in alcoholics due to poor nutrition. Folic acid deficiency is more common than vitamin B_{12} deficiency because body stores of folic acid are not durable. However, vitamin B_{12} deficiency must also be ruled out as it may arise with or without a concurrent folate deficiency. Hypovitaminosis B_{12} may arise from poor nutrition but is more commonly caused by pathologies such as pernicious anemia. It is critical that both serum folate and vitamin B_{12} concentrations be drawn in this patient to guide appropriate supplementation. Replenishment of folate in a patient with hypovitaminosis B_{12} may temporarily improve the values of the hemogram, but failure to appropriately replenish vitamin B_{12} can lead to irreversible brain and nerve damage.

homologous with intrinsic factor.[16] If impaired, there are several steps in the absorption of vitamin B_{12} that may be responsible for a deficiency.

A deficiency of cobalamin (vitamin B_{12}) may arise from inadequate intake of the vitamin or from a deficiency of the intrinsic factor required for the effective ileal absorption of the vitamin. Inadequate dietary intake is a rare cause of vitamin B_{12} deficiency, usually occurring only in vegans who abstain from all animal food including milk and eggs.[14] On the other hand, defective production of intrinsic factor is a common cause of the deficiency.[17] The gastric mucosa can fail to secrete intrinsic factor because of atrophy, especially in the elderly.

Clinical and laboratory diagnosis. Vitamin B_{12} is necessary for deoxyribonucleic acid (DNA) synthesis in all cells, for the synthesis of neurotransmitters, and for metabolism of homocysteine. Therefore, B_{12} deficiency leads to signs and symptoms involving many organ systems.[14,15] The most notable symptoms involve the following:

- Gastrointestinal (GI) tract (e.g., loss of appetite, smooth and sore tongue, and diarrhea or constipation)
- Central nervous system (e.g., paresthesias in fingers and toes, loss of coordination of legs and feet, tremors, irritability, somnolence, and abnormalities of taste and smell)
- Hematopoietic system (anemia)

Nuclear maturation retardation occurs in the developing cells in the bone marrow due to slowed DNA synthesis. The morphological result—cells with an immature and enlarged nuclei (megaloblasts) but a cytoplasm that matures normally—causes mature cells to be larger than normal. The resulting anemia is called a *macrocytic*, *megaloblastic anemia,* which has both morphological characteristics of nuclear maturation retardation.[15] Visual inspection of smears of both peripheral blood and bone marrow reveals characteristic megaloblastic changes in the appearance of erythrocytes and WBCs. The development of neutrophils is also affected, which results in large cells with hypersegmentation (greater than three nuclear lobes). A mild pancytopenia (decreased numbers of all blood elements) also occurs. The usual laboratory test results found with vitamin B_{12} deficiency are listed in Table 16-2.

In the past, serum cobalamin (vitamin B_{12}) concentrations were measured using a microbiologic assay and a cobalamin-dependent organism. The assay has largely been replaced by a competitive displacement assay using radioactive cobalamin and intrinsic factor. Unfortunately, because of cross-reactivity with other cobamides, approximately 5% of patients will have cobalamin concentrations that appear to be within the normal range yet can be shown to have hematologic/neurologic signs of deficiency. There is increasing acceptance of the use of serum levels of the metabolic intermediates homocysteine and methylmalonate as more sensitive indicators of cobalamin deficiency. In the presence of inadequate cobalamin, these two compounds accumulate because of the cobalamin dependence of their metabolizing enzymes, methionine synthase and methylmalonyl-CoA-mutase, respectively. An elevated serum methylmalonate and plasma homocysteine concentration in the presence of normal RBC folate is strongly indicative of a pure deficiency of cobalamin.

Historically, the Schilling test was used to determine if impaired absorption is the reason for the cobalamin deficiency. In the Schilling test, cyano-(^{57}Co)-cobalamin is administered orally and is allowed to be absorbed, if possible. An unlabeled, intravenous (IV) injection of cobalamin follows and will displace some radioactive ^{57}Co-cobalamin from transcobalamin that is excreted and measured in the urine. If oral absorption is normal (e.g., the deficiency was caused by inadequate dietary intake), >8% of the radioactive cobalamin will be excreted in the urine. If abnormal, the test can be repeated at a later date (stage II test), adding exogenous intrinsic factor to the dose of oral labeled CN-(^{57}Co)-cobalamin to determine if inadequate urinary excretion secondary to poor absorption was due to a relative lack of the protein.[15] The availability of intramuscular injections of vitamin B_{12} obviates the need to specify the defect in B_{12} absorption, and such injections are favored in patients with impaired cobalamin absorption, regardless of the cause. Oral cobalamin can also be used in most patients with intrinsic factor deficiency, but regular monitoring is required to ensure efficacy and adherence to dosing. The availability of cobalamin supplementation and the complexity of the procurement and disposal of radioactive tracers discourage the use of the Schilling test.[15]

Pernicious anemia is a separate disease characterized by atrophic gastritis associated with antibodies against intrinsic factor and gastric parietal cells. Gastrectomy (removal of all or part of the stomach) can also lead to vitamin B_{12} deficiency because the procedure removes the production site of intrinsic factor. Achlorhydria from gastrectomy or drugs such as proton pump inhibitors can decrease the release of cobalamin from meat. Defective or deficient absorption of the intrinsic factor–vitamin B_{12} complex can be caused by inflammatory disease of the small bowel, ileal resection, and bacterial overgrowth in the small bowel.[16,18,19] Administration of colchicine, neomycin, and para-aminosalicylic acid can also lead to impaired absorption of vitamin B_{12} (Table 16-3).

Folic Acid Deficiency

Folic acid is also called *pteroylglutamic acid*. Folates refer to folic acid or reduced forms of folic acid that may have variable numbers of glutamic acid residues attached to the folic acid molecule. The folates present in food are mainly in a polyglutamic acid form and must be hydrolyzed in the intestine to the monoglutamate form to be absorbed efficiently. The liver is the chief storage site. Adult daily requirements are approximately 50 mcg of folic acid, equivalent to about 400 mcg of food folates. Folate stores are limited, and anemia arising from a folate-deficient diet occurs in four to five months.[14]

Inadequate dietary intake is the major cause of folate deficiency. Folates are found in green, leafy vegetables such as spinach, lettuce, and broccoli. Inadequate intake can have numerous causes: alcoholics often have poor nutritional intake of folic acid; certain physiological states such as pregnancy require an increase in folic acid; malabsorption syndromes (mentioned in the section on vitamin B_{12}) can lead to defective absorption of folic acid; and celiac sprue can lead to folate malabsorption.[18]

Certain medications (e.g., methotrexate, trimethoprim–sulfamethoxazole, and triamterene) can act as folic acid antagonists by interfering with the conversion of folic acid into its metabolically active form, tetrahydrofolic acid. Phenytoin and phenobarbital administration can interfere with the intestinal absorption or utilization of folic acid (Table 16-3).[20]

Folic acid is required as the intermediate for one-carbon transfers in several biochemical pathways, including the thymidine required for DNA synthesis. After absorption, folate is reduced to tetrahydrofolate, and a carbon in one of several oxidation states is attached for transfer. Most transfer processes allow facile regeneration. However, the formation of methyltetrahydrofolate requires vitamin B_{12} as a cofactor for the methyl group transfer. Methyltetrahydrofolate is required for the conversion of homocysteine to methionine, which is subsequently used as a methyl donor in many synthetic pathways that include the production of critical neurotransmitters and amino acids.

Clinical and laboratory diagnosis. Because folic acid is necessary for DNA synthesis, a deficiency causes a maturation retardation in the bone marrow similar to that caused by vitamin B_{12} deficiency. Folic acid deficiency is also characterized by a macrocytic, megaloblastic anemia.[16] However, with folic acid deficiency, pancytopenia does not develop as consistently as it does with vitamin B_{12} deficiency.

Folate supplementation in patients with a folate deficiency will provide folate for the nonmethyl transfer steps that do not require vitamin B_{12}. These processes include RNA and DNA synthesis and can often, at least partially, reverse megaloblastic anemia. However, without adequate vitamin B_{12}, the lack of methionine synthesis will lead to potentially serious and irreversible neurological damage. It is not yet clear whether this damage is due to a deficiency in the methionine-dependent neurotransmitters and amino acids or to accumulation of homocysteine. Although folate deficiency is more common and easily treated, it is critical to correctly identify the cause of a megaloblastic anemia so that any vitamin B_{12} deficiency is appropriately treated.

Folate Concentration

Normal range: serum folate 5–25 mcg/L (11.33–56.65 nmol/L)
RBC folate 166–640 mcg/L (376.16–1450.24 nmol/L)

The *folate concentration* in both serum and in erythrocytes (RBCs) is used to assess folate homeostasis. A low serum folate indicates negative folate balance and can be expected to lead to folate deficiency when hepatic folate stores are depleted.

Microcytic Anemia

Iron Deficiency

Microcytic anemia, or anemia with abnormally small erythrocytes is typically caused by iron deficiency. Decreased MCV is a late indicator of the deficiency (Figure 16-2). Daily requirements are approximately 1 mg of elemental iron for each 1 mL of RBCs produced, so daily iron requirements are approximately 20–25 mg for erythropoeisis.[21] Most iron needed within the body is obtained by recycling metabolized Hgb. RBCs have a lifespan of approximately 120 days. When old or damaged erythrocytes are taken up by macrophages in the liver, spleen, and bone marrow, the Hgb molecule is broken down and iron is extracted and stored with proteins. Only about 5% of the daily requirement (1 mg) is newly absorbed to compensate for losses due to fecal and urinary excretion, sweat, and desquamated skin. (**Minicase 2**.)

Menstruating women require more iron because of increased blood losses. Iron requirements vary among women but averages 2 mg/day. Orally ingested iron is absorbed in the GI tract, which should permit just enough iron absorption to prevent excess or deficiency. Typically, 5–10% of oral intake is absorbed (normal daily dietary intake: 10–20 mg).[21]

Iron deficiency is usually due to inadequate dietary intake and increased iron requirements. Poor dietary intake, especially in situations that require increased iron (e.g., pregnancy), is a common cause. Other causes of iron deficiency include the following:

- Blood loss due to excessive menstrual discharge
- Peptic ulcer disease
- Hiatal hernia
- Gastrectomy
- Gastritis due to the ingestion of alcohol, aspirin, and nonsteroidal anti-inflammatory drugs (NSAIDs)
- Bacterial overgrowth of the small bowel
- Inflammatory bowel disease
- Occult bleeding from GI carcinoma
- Starch or clay pica

Ionized, soluble iron is toxic because of its ability to mediate the formation of oxidative species. Iron is therefore bound to proteins both in and outside of cells. The iron-protein complex within the macrophage is known as *ferritin* (**Figure 16-3**). In the normal adult, approximately 500–1500 mg is stored as ferritin and 2500 mg of iron is contained in Hgb.[21] When the total quantity of extracted iron exceeds the amount that can be stored as ferritin, the excess iron is stored in an insoluble form called *hemosiderin*.

Although ferritin is primarily stored in macrophages, small amounts can be found in plasma and can be measured. Therefore, serum ferritin concentration reflects total body iron stores and is the most clinically useful method to evaluate patients for iron deficiency. Because the protein is an acute phase reactant, serum ferritin concentrations can be increased by chronic infections, fever, and inflammatory reactions. The transport of iron in plasma and extracellular fluid occurs with two ferric ions bound to the protein transferrin, which when not binding iron or other metals is termed *apotransferrin*. Transferrin binds to specific membrane transferrin receptors where the complex enters the cell and releases the iron. Apotransferrin is released when the apotransferrin-receptor complex returns to the surface of the cell.

The tendency of ferritin to be falsely elevated with inflammatory processes has led to recent interest in using soluble

MINICASE 2

Anemia and Iron Stores

Denise T. is a 25-year-old woman seen in a community health clinic for a routine checkup. Her family history includes a sister with sickle cell disease. She has not been affected personally but has not been tested to determine her sickling genotype. She describes painful menstrual periods and takes aspirin for them. She also admits to a pica of ingesting cornstarch throughout the day. The following laboratory tests are obtained:

QUESTION: What hematologic abnormalities are apparent from these results?

DISCUSSION: This patient demonstrates an anemia as manifested by the decreased hematocrit, hemoglobin, and RBC. Her WBC and platelet counts are normal. The RDW is elevated, indicating increased variability of erythrocyte size (anisocytosis). Because the MCV is low, we can presume that this microcytic, hypochromic form of anemia is most likely due to rather prolonged iron deficiency. This is corroborated by the iron studies, which indicate a low serum iron and transferrin saturation. Serum ferritin is also decreased, indicating that her iron stores are markedly reduced. The TIBC is increased both because of increased transferrin production and decreased iron available to bind to the protein.

There may be multiple causes of her iron deficiency. Most commonly, the combination of low dietary iron and blood loss from menstruation increases the frequency of iron deficiency anemia in women. An additional possibility is occult blood loss from gastrointestinal ulcerations caused by aspirin. An exacerbating factor for this woman is her starch pica, or craving for unusual food. In addition to the high caloric intake associated with this particular pica, the starch decreases the bioavailability of ingested iron, decreasing the ability of the patient to absorb dietary or supplemental iron. Given the pica, parenteral iron may be considered.

TEST NAME	RESULT	REFERENCE RANGE
RBC	3.3 × 10^6 cells/μL	4.1–5.1 × 10^6 cells/μL for females
WBC	5.1 × 10^3 cells/μL	4.4–11.3 × 10^3 cells/μL
Hgb	8.3 g/dL	12.3–15.3 g/dL for females
Hct	26%	36–45% for females
MCV	78 fL/cell	80–96 fL/cell
RDW	16.1%	11.5–14.5%
Platelet count	195,000 cells/μL	150,000–450,000 cells/μL
Neutrophils	52%	45–73%
Bands	3%	3–5%
Monocytes	2%	2–8%
Eosinophils	1%	0–4%
Basophils	0%	0–1%
Lymphocytes	42%	20–40%
Serum iron	44 mcg/dL	50–150 mcg/dL
TIBC	451 mcg/dL	250–410 mcg/dL
Transferrin saturation	14%	30–50%
Serum ferritin	5.2 mcg/L	10–20 mcg/L

transferrin receptor concentrations as an alternative marker of iron deficiency. The circulating receptor fragment is considered to reflect total body receptor expression and is elevated in times of increased erythropoiesis such as sickle cell anemia, thalassemias, and chronic hemolysis. If such causes of increased erythropoiesis can be excluded, elevated concentrations of circulating transferrin receptor are thought to reflect iron deficiency. The use of transferrin receptor concentrations may help determine if decreased ferritin concentrations are due to iron deficiency or to anemia of chronic (inflammatory) disease. Elevated plasma concentrations of ferritin can be caused by rheumatoid arthritis, various malignancies, hepatitis, or infections.

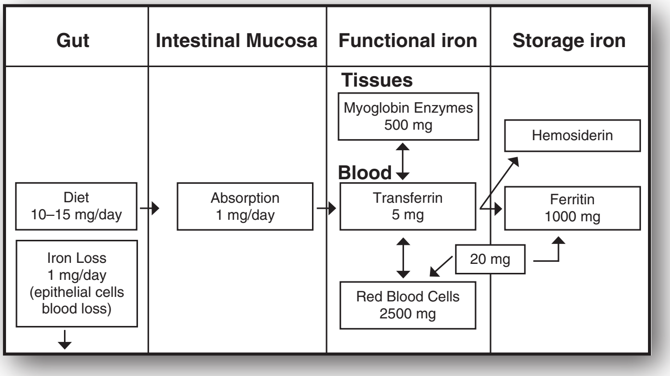

FIGURE 16-3. Intake, loss, and recycling of iron and iron storage forms.

Clinical and laboratory diagnosis. The first change observed in the development of iron deficiency anemia is a loss of storage iron (hemosiderin). If the deficiency continues, a loss of plasma iron occurs. The decrease in plasma iron stimulates an increase in transferrin synthesis. When enough iron has been depleted such that supplies for erythropoiesis are inadequate, anemia develops. The RDW will rise, often before the MCV decreases to a notable degree. If the iron deficiency persists, the RBCs become smaller than usual (microcytic—low MCV) and not as heavily pigmented as normal RBCs because they contain less Hgb than normal erythrocytes (hypochromic-low MHC and MCHC). Clinically, patients present with progressively worsening weakness, fatigue, pallor, shortness of breath, tachycardia, and palpitations. Numbness, tingling, and glossitis also may exist.[10] Laboratory results for iron deficiency anemia are listed in Table 16-2. With adequate iron therapy the maximal daily rate of Hgb regeneration is 0.3 g/dL, or approximately 1%/day in Hct.

Serum Ferritin

Normal range: >10–200 ng/mL (>10–200 mcg/L)

Loss of storage iron (hemosiderin) was traditionally evaluated by iron-stained bone marrow aspirate. *Serum ferritin* has largely replaced these invasive tests as an indirect measure of iron stores. Serum ferritin concentrations are markedly reduced in iron deficiency anemia (3–6 mcg/L).

Serum Iron and Total Iron-Binding Capacity

Serum iron normal range: 50–150 mcg/dL (9–26.9 μmol/L)
TIBC normal range: 250–410 mcg/dL (44.8–73.4 μmol/L)

The *serum iron* concentration measures iron bound to transferrin. This value represents about one third of the *total iron-binding capacity* (TIBC) of transferrin.[21] The TIBC measures the iron-binding capacity of transferrin protein. In iron deficiency anemia, TIBC is increased due to a compensatory increase in transferrin synthesis.[22] This increase leads to a corresponding decrease in the percent transferrin saturation that can be calculated by dividing the serum iron by the TIBC and then multiplying by 100. For example, a person with a serum iron concentration of 100 mcg/dL and a TIBC of 300 mcg/dL has a transferrin saturation of 33%. Iron deficient erythropoiesis exists whenever the percent saturation is 15% or less.

Other disease states besides iron deficiency that can alter serum iron and TIBC are infections, malignant tumors, and uremia.[9,10] Anemia from these diseases is sometimes called *anemia of chronic disease.* Serum iron and TIBC both decrease in these disorders, unlike in iron deficiency anemia where serum iron decreases but TIBC increases. (Minicase 2.)

Normochromic, Normocytic Anemia

This classification encompasses numerous etiologies. Three causes are discussed: acute blood loss anemia, hemolytic anemia, and anemia of chronic disease.

Acute Blood Loss Anemia

Patients who suffer from acute hemorrhage may experience a dramatic drop in their whole blood volume. In this situation, the Hct is not a reliable indicator of the extent of anemia. It is a measure of the amount of packed RBCs per unit volume of the blood, not the total body amount of RBCs. The total whole blood volume may be markedly reduced, but in the acute phase of the hemorrhage the Hct may be normal or even slightly increased. Hemorrhage evokes vasoconstriction which initially prevents extravascular fluid from replacing intravascular fluid loss. Usually, both Hgb and Hct are decreased by the time that a CBC is obtained. This is also evident after the administration of crystalloid IV fluids to maintain intravascular volume, an example of an iatrogenic drop in RBC.

In patients with normal bone marrow, the production of RBCs increases in response to hemorrhage, resulting in reticulocytosis. If the patient is transfused, each unit of packed RBCs administered should increase the Hgb by 1 g/dL if the bleeding has stopped. Table 16-2 shows the usual laboratory findings in acute blood loss anemia. (**Minicase 3.**)

MINICASE 3

Anemia and Low Platelet Count

Michael T., a 50-year-old male with a long history of alcohol abuse, cirrhosis, and esophageal varices, is brought to the emergency department (ED) by concerned family members. The family says that he suddenly began coughing up bright red blood. As he is moved to a bed in the ED, he begins coughing and vomiting large amounts of bright red blood. A stat CBC reveals the following:

TEST NAME	RESULT	REFERENCE RANGE
RBC	2.91×10^6 cells/μL	4.5–5.9 × 10^6 cells/μL for males
WBC	6.6×10^3 cells/μL	4.4–11.3 × 10^3 cells/μL
Hgb	8 g/dL	14–17.5 g/dL for males
Hct	28.2%	42–50% for males
MCV	92.4 fL/cell	80–96 fL/cell
RDW	14.1%	11.5–14.5%
Platelet	75,000 cells/μL	150,000–450,000 cells/count μL

QUESTION: What does this CBC indicate?

DISCUSSION: The presence of bright blood (as opposed to dark, "coffee ground" material) in the emesis indicates an acute and active bleed—either from a gastric ulcer or from esophageal varices. The CBC is consistent with acute blood loss. At the onset of bleeding, the RBC, Hgb, and Hct may show minimal changes. Here, the RBC, Hgb, and Hct are all moderately decreased, and the red cell indices are within normal limits, supporting a recent history of significant blood loss. The platelet count is also decreased, which may have led to the increasing blood loss. As could be anticipated from his history of alcohol abuse, the bleeding was found to arise from a ruptured esophageal varix.

Hemolytic Anemia

Hemolysis is the lysis of erythrocytes. Hemolysis often leads to irregularly shaped or fragmented erythrocytes, termed *poikilocytosis*. If hemolysis is rapid and extensive, severe anemia can develop, yet RBC indices (MCV and MCHC) remain unchanged in the short term. Patients with normal bone marrow respond with an increase in erythrocyte production to replace the lysed cells, and reticulocytosis is present. Specialized tests, called *antiglobulin tests*, can be useful in determining immune causes of hemolytic anemia.[23]

Plasma (free) Hgb measures the concentration of Hgb circulating in the plasma unattached to RBCs. It is almost always elevated in the presence of intravascular hemolysis. Haptoglobin, an acute-phase reactant, binds free Hgb and carries it to the reticuloendothelial system. In the presence of intravascular hemolysis, haptoglobin is decreased. Concomitant corticosteroid therapy may confound interpretation because many diseases associated with in vivo hemolysis are treated with steroids. Serum haptoglobin may be normal or elevated in hemolysis if the patient is receiving steroids. If the increase in serum haptoglobin is from steroids, other acute-phase reactants such as prealbumin or ferritin will also be elevated. Serum haptoglobin is also elevated in patients with biliary obstruction and nephrotic syndrome. It is variably decreased in folate deficiency, sickle cell anemia, thalassemia, hypersplenism, liver disease, estrogen therapy, and pregnancy.[8]

Immune hemolytic anemias are caused by the binding of antibodies and complement components to the erythrocyte cell membrane with subsequent lysis.[24] The method used to detect autoantibodies already bound to erythrocytes is a direct antiglobulin test (DAT), sometimes referred to as the *direct Coombs test*. The method used to detect antibodies present in serum is an indirect antiglobulin test (IAT, indirect Coombs). The DAT is performed by combining a patient's RBCs with rabbit or goat antihuman globulin serum, which contains antibodies against human immunoglobulins and complement.[22] If the patient's RBCs are coated with antibody or complement, the antibodies in the antiglobulin serum bind to the immunoglobulins coating the RBCs, leading to the agglutination of the RBCs. The DAT is the only test that provides definitive evidence of immune hemolysis.[23] The DAT can also be used to investigate possible blood transfusion reactions.[22]

The IAT detects antibodies in the patient's serum. Patient serum is combined with several types of normal erythrocytes of known antigenic expression. Any antibodies able to bind to the antigens expressed on these sample RBCs will adhere after the serum is washed away. Antihuman immune globulin is then added and will bind to any of the patient's immune globulin that is present on the erythrocytes, followed by agglutination.[22,24]

The antiglobulin tests are very sensitive, but a negative result does not eliminate the possibility of antibodies bound to erythrocytes. An estimated 100–150 molecules of antibody must be bound to each erythrocyte for detection by the antiglobulin test.[22] Smaller numbers of antibodies give a false-negative reaction. Numerous conditions and medications can be associated with immune hemolytic anemia (Table 16-4)[24] Medications can induce antibody formation by three mechanisms that result in a hemolytic anemia.

Autoimmune type. Methyldopa and procainamide are infrequently used cardiovascular drugs that may induce the formation of antibodies directed specifically against normal RBC proteins. This autoimmune state can persist for up to one month after drug administration has been discontinued. This mechanism is known as a true autoimmune type of antibody formation and is detected using the DAT.[25]

Innocent bystander type. Antibodies to the drugs quinine and quinidine are examples of the immune complex (innocent bystander) mechanism.[24] Each drug forms a drug-protein complex with plasma proteins to which antibodies are formed. This drug-plasma protein-antibody complex attaches to erythrocytes and fixes complement, which leads to lysis of the RBCs.[22] In this situation, the RBC is an innocent bystander. Examples of other drugs implicated in causing this type of hemolytic anemia are listed in **Table 16-4**.

TABLE 16-4. Causes of Immune Hemolytic Anemia

Neoplasm
Chronic lymphocytic leukemia
Lymphoma
Multiple myeloma
Collagen vascular disease
Systemic lupus erythematosus
Rheumatoid arthritis
Medication
Autoimmune type
Levodopa, mefenamic acid, methyldopa, procainamide
Innocent bystander type
Cefotaxime, ceftazidime, ceftriaxone, chlorpromazine, doxepin, fluorouracil, isoniazid, quinidine, quinine, rifampin, sulfonamides, thiazides, chlorpropamide
Hapten type 1
Cephalosporins, penicillins
Infections
Mycoplasma
Viruses

Hapten type 1. The hapten (penicillin) type 1 mechanism is involved when a patient has produced antibodies to penicillin. If the patient receives penicillin at a future date, some penicillin can bind to the RBC membrane. The antipenicillin antibodies, in turn, bind to the penicillin bound to the RBC, and hemolysis can result.

G6PD Deficiency Anemia

Glucose-6-phosphate dehydrogenase (G6PD) is an intracellular enzyme that forms the nicotinamide adenine dinucleotide phosphate needed by the erythrocyte to synthesize the antioxidant glutathione. Variants of this enzyme are more commonly found in African Black (Gd^{A-}), Mediterranean, and Asian populations (Gd^{Med}) than in Caucasians. These variants have an impaired ability to resist the oxidizing effect of drugs and of collateral oxidative exposure to the granulocytic response to infections. Thus, exposure of patients with G6PD deficiency to oxidizing drugs or to an infection can lead to a dramatic, nonimmunologic hemolysis. Examples of drugs that can lead to hemolysis in G6PD-deficient patients include dapsone, primaquine, rasburicase, phenazopyridine, methylene blue, and nitrofurantoin.[27] Drug-induced hemolysis is less likely in the Gd^{A-} variant, but both variants are susceptible to infection-induced hemolysis.[28] Assessment of at-risk patients for signs of hemolysis (anemia, hemoglobinemia, dark urine, and back pain) is appropriate. Routine genotyping of patients will aid in drug selection and monitoring of at-risk populations.

Anemia of Chronic Disease

Mild-to-moderate anemia often accompanies renal failure, various infections, inflammatory traumatic illnesses, or neoplastic diseases that last over one to two months.[29] Chronic infections include pulmonary abscesses, tuberculosis (TB), endocarditis, pelvic inflammatory disease, and osteomyelitis. Chronic inflammatory illnesses (e.g., rheumatoid arthritis and systemic lupus erythematosus) and hematological malignancies (e.g., Hodgkin disease, leukemia, and multiple myeloma) are also associated with anemia. Because these disorders as a group are common, anemia due to chronic disease is also quite common. Although anemia of chronic disease is more commonly associated with normocytic, normochromic anemia, it can also cause microcytic anemia. Table 16-2 shows the usual laboratory results found in anemia of chronic disease.

The pathogenesis of this anemia is not totally understood. Various investigations have found that the erythrocyte lifespan is shortened and that the bone marrow does not increase erythrocyte production to compensate for the decreased longevity. Iron utilization is also impaired. Although erythrocytes are frequently normal size, microcytosis can develop. One distinguishing feature between early iron deficiency anemia and microcytic anemia of chronic disease is the normal serum ferritin that is present in the latter.[29]

In patients with anemia associated with chronic kidney disease, iron supplementation is recommended for those with ferritin concentrations <100 mcg/L. If anemia is present but ferritin concentrations are >100 mcg/L, the need for iron is demonstrated by a transferrin saturation of <20%.[30] In the presence of sufficient iron stores, erythrocyte-stimulating agents (ESA) such as recombinant erythropoietin and darbepoetin may be used to decrease a patient's need for blood cell transfusions. The U.S. Food and Drug Administration (FDA) has modified recommendations for more conservative dosing of ESAs due to recent data suggesting ESAs increase the risk for serious adverse cardiovascular events. A target hemoglobin range is not provided in the recommendations. Instead, dosing should be individualized to use the lowest dose of ESA sufficient to reduce the need for transfusion. Patients with chronic kidney disease (CKD) who are not on dialysis should consider starting ESA treatment only when the hemoglobin level is <10 g/dL and reduce or stop the ESA dose if the hemoglobin level exceeds 10 g/dL. For patients on dialysis, ESA treatment should be initiated when the hemoglobin level is <10 g/dL and reduce or interrupt the ESA dose if the hemoglobin level approaches or exceeds 11 g/dL. Monitoring of hemoglobin levels should be done at least weekly until stable and then monitored monthly.[31]

Use of ESA therapy in patients with cancer has become controversial due to the increased risk of thromboembolism and shorter survival.[32] ESA therapy only is recommended in patients undergoing myelosuppressive chemotherapy who have a hemoglobin of <10 g/dL. As with CKD patients, dosing should be individualized to use the lowest dose of ESA sufficient to reduce the need for transfusion. Use of ESAs is not recommended for cancer patients in any other circumstances. Because of the potential for adverse events, the FDA has required participation of prescribers, pharmacies, and patients in a Risk Evaluation and Mitigation Strategy for all erythropoietic agents.[33]

Hemoglobinopathies

Several diseases arise from abnormal synthesis of the α or β subunits of hemoglobin. The most common types of anemias related to these *hemoglobinopathies* include sickle cell trait/disease and thalassemias. Sickle cell trait is caused by the substitution of a valine amino acid for glutamate on the β chain of hemoglobin. The heterozygous carrier state is thought to provide a resistance to clinical manifestations and sequelae of malaria. Homozygous persons with both β chains carrying the valine substitution are at increased risk of developing a sickling of erythrocytes. This occurs most commonly under circumstances of hypoxia, infection, dehydration, or acidosis. Deoxygenated hemoglobin molecules polymerize into rod-like structures within the RBC, deforming the cell into an arched, rigid sickled cell. These erythrocytes are not able to deform and pass through the capillaries or reticuloendothelial system. Hypoxia, ischemia, and even infarction occur in tissues downstream of these sites of impaired erythrocyte flow. Severe pain is usually present during these "sickle crises," and opiate analgesics are often needed in addition to hydration, transfusion, and other treatments. Diagnosis is made by inspection of the peripheral blood smear and by electrophoresis of the patient's hemoglobin.[34,35]

Thalassemias are a more diverse group of hemoglobinopathies most commonly associated with persons of ancestry arising in the Mediterranean region. Unlike the chemical change caused by the valine substitution in sickle cell patients, thalassemias are characterized by a deficiency or absence of one of the subunits of the hemoglobin. Because there are two α and two β hemoglobin subunits in the normal hemoglobin tetramer, an inability to produce adequate amounts of one of the subunits would clearly lead to difficulty in synthesizing intact, complete hemoglobin molecules.[36]

Thalassemias are often diagnosed by a peripheral blood smear, which shows small, pale erythrocytes. Some of the RBCs are nucleated, reflecting the intense pressure on erythropoiesis in the bone marrow to provide oxygen carrying capacity to the body even if it requires releasing immature, nucleated erythrocyte precursors. The type of thalassemia present is determined using electrophoresis.

WHITE BLOOD CELL COUNT AND DIFFERENTIAL

WBCs are divided into two general classifications:

1. Granulocytes or phagocytes (leukocytes that engulf and digest other cells)
2. Lymphocytes (leukocytes involved in the recognition of nonself cells or substances)

The functions of these general leukocyte classes are interrelated. For example, immunoglobulins produced by B lymphocytes are needed to coat or opsonize encapsulated bacteria so that T cells and neutrophils can more effectively identify, adhere, and destroy them.

TABLE 16-5. Normal WBC Count and Differential

CELL TYPE	NORMAL RANGE
Total WBC count	4.4–11.3 × 10^3 cells/µL
Polymorphonuclear neutrophils ("polys," "segs," PMN)	45–73%
Band neutrophils ("bands," "stabs")	3–5%
Lymphocytes	20–40%
Monocytes	2–8%
Eosinophils	0–4%
Basophils	0–1%

PMN = polymorphonuclear cells; WBC = white blood cell.

When a WBC count and differential is ordered for a patient, the resulting laboratory report is a tally of the total WBCs in a given volume of blood plus the relative percentages each cell type that contribute to the total. Therefore, the percentages of the WBC subtypes must add up to 100%. If one cell type increases or decreases, percentages of all other types will be expected to change somewhat in the opposite direction. **Table 16-5** is a general breakdown of the different types of WBCs and their usual percentages in peripheral blood.

The WBC count and differential is one of the most widely performed clinical laboratory tests. In the past, differentials were determined by a manual count of a standard number of cells. In addition to being labor-intensive and slow, this method is imprecise and inaccurate when compared to automated methods.[37] Large, clinical laboratories commonly use automated methods for determining the WBC differential, but manual differential counts are still common. Automated instruments count thousands of cells and can report not only the relative percentages of the various WBC types but also the absolute numbers, Hgb, RBC, platelets, and RBC indices. When reviewing a WBC differential, one must be aware of not only the relative percentages of cell types but also the absolute numbers. The percentages viewed in isolation can lead to incorrect conclusions. **Minicase 4** demonstrates this principle.

Numerous cluster of differentiation (CD) surface markers have been characterized on leukocytes and their precursors. CD molecules are surface proteins or glycoproteins that are typically immunologically characterized by their unique epitopes. The function of only a minority of the hundreds of CD molecules identified on human cells have been determined; however, their expression on specific cell types can permit identification of abnormal cell types and allow targeted treatment at cells expressing the CD molecule.

MINICASE 4

Blast Crisis

David D., a 46-year-old male, presents to the emergency department with a temperature of 104 °F (40 °C), diarrhea, and abdominal pain. Urine and blood cultures are obtained, and he is given broad spectrum antibiotics. The following CBC is obtained:

TEST NAME	RESULT	REFERENCE RANGE
RBC	3.18 × 10^6 cells/µL	4.5–5.9 × 10^6 cells/µL for males
WBC	118.9 × 10^3 cells/µL	4.4–11.3 × 10^3 cells/µL
Hgb	9.9 g/dL	14–17.5 g/dL for males
Hct	29.5%	42–50% for males
MCV	92.8 fL/cell	80–96 fL/cell
RDW	14.1%	11.5–14.5%
Platelet	69,000 cells/µL	150,000–450,000 cells/ count µL
Neutrophils	21%	45–73%
Bands	12%	3–5%
Metamyelocytes	5%	0%
Myelocytes	5%	0%
Promyelocytes	8%	0%
Lymphocytes	6%	20–40%
Atypical	0%	0% lymphocytes
Monocytes	2%	2–8%
Eosinophils	1%	0–4%
Basophils	10%	0–1%
Blasts	30%	0%

QUESTION: What does his CBC reveal?

DISCUSSION: This CBC is grossly abnormal, showing marked leukocytosis with elevations in the absolute neutrophil count, neutrophil precursors, and blast cells. Although the percentage of neutrophils is decreased, a neutrophilia is revealed when the absolute number of neutrophils is calculated (e.g., 118.9 × 10^3 × 21% = 25,000 cells/µL; normal range 2200–8000 cells/µL). Bands are also neutrophilic leukocytes and may be included in the absolute neutrophil count at some clinical sites. He has a normochromic, normocytic anemia and thrombocytopenia.

At first, one might expect that his condition could be consistent with an overwhelming infection. However, he has a marked number of immature WBC forms in the peripheral blood—metamyelocytes, myelocytes, promyelocytes, and particularly blasts. These forms are normally found only in the bone marrow and not in the circulation.

A bone marrow biopsy reveals he has chronic myelogenous leukemia with a blast (myeloblast) crisis. The anemia is likely myelophthisic, which occurs in part by the "crowding out" of maturing RBCs and WBCs in the bone marrow by the neoplastic immature WBCs including blast cells. This would also result in a normochromic, normocytic anemia.

Granulocytes

Granulocytes are phagocytes (eating cells) and derive their name from the presence of granules within the cytoplasm. The granules store lysozymes and other chemicals needed to oxidize and enzymatically destroy foreign cells. Granulocytic leukocytes include neutrophils, eosinophils, and basophils. Monocytes are phagocytic cells that mature into macrophages, which are predominantly found in tissue rather than in the circulation. When a peripheral smear of blood is prepared, three types of granulocytes are named by the staining characteristics of their cytoplasmic granules[8]:

1. Neutrophils retain neutral stains and appear light tan.
2. Eosinophils retain acidic dyes and appear red-orange.
3. Basophils retain basic dyes and appear dark blue to purple.

Granulocytes are formed in large numbers from the pluripotential stem cells in the bone marrow. They undergo numerous differentiation and proliferation steps in the marrow and are usually released into the peripheral blood in their mature form. A common exception is the appearance of band cells during an infection, as discussed below. Neutrophils, eosinophils, and basophils die in the course of destroying ingested organisms or particles, yielding pus. On the other hand, monocytes and macrophages do not usually need to sacrifice themselves when destroying target cells.

Neutrophils

Normal range: PMN leukocytes 45–73% or 0.45–0.73
bands 3–5% or 0.03–0.05

Neutrophils are also termed *segmented neutrophils* (or "segs") or *polymorphonuclear cells* (PMNs or "polys"). The less mature form of the neutrophil with a crescent-shaped nucleus is a band or stab cell. Bands derive their name from the morphology of their nucleus, which has not yet segmented into multiple lobes. Less mature forms of the neutrophil, such as the metamyelocyte and myelocyte, are normally not in the peripheral blood. The neutrophil is a phagocytic cell that exists to ingest and digest foreign cells and proteins (e.g., bacteria and fungi).

The absolute segmented neutrophil count is the percentage of neutrophils and bands multiplied by the WBC count. The reference range for absolute counts can be estimated by multiplying the normal range of percentages for the particular type of WBC by the upper and lower limits of the total WBC count. Absolute neutrophil counts of <1000/ μL represent neutropenia, with counts of <500/μL and 100/μL considered severe and absolute neutropenia, respectively. Because of the risk of rapidly progressing, life-threatening infection, prophylactic antibiotics are typically started after cytotoxic chemotherapy if the absolute neutrophil count is expected to be <1000/μL for more than seven days.[38]

Under normal conditions, about 90% of the neutrophils are stored in the bone marrow. When released, neutrophils will normally circulate for several hours before eventually marginating and rolling along the endothelium until finally stopping and adhering. This dynamic process of margination, with the potential for demargination causes large shifts in the measured neutrophil count, because only the granulocytes that are circulating at the time are measured by a venipuncture. Neutrophils spend only about six to eight hours in the circulation after which they move through the endothelium into the tissue. Unless used to engage a foreign body or sustained by the cytokine milieu, neutrophils then undergo programmed cell death, a noninflammatory process termed *apoptosis.*[39]

During an acute infection there is an increase in the percentage of neutrophils as they are released from the bone marrow and demarginate from the endothelium.[40,41] Less mature band forms may also be released, but these immature neutrophils are still considered to be active. The appearance of band cells in infections is termed a *left shift*. This may be due to the traditional order in which the differential was reported. It may also arise from the use of a left-to-right sequence in figures describing the process of neutrophil differentiation from the stem cell (Figure 16-1).

When the neutrophils and bands are elevated, the percentage of lymphocytes usually decreases. Ratios of only 10–15% lymphocytes may appear in these patients, but this relative lymphopenia arises from the concomitant increase in total WBCs. An exception is a neutrophilia caused by glucocorticoid treatment, which will cause a drop in the absolute lymphocyte count because of its lymphotoxic effect while increasing the absolute neutrophil count due to demargination.

Eosinophils and Basophils

Normal range: eosinophils 0–4% or 0–0.04;
basophils 0–1% or 0–0.01

The functions of *eosinophils* and *basophils* are not completely known. Eosinophils are present in large numbers in the intestinal mucosa and lungs, two locations where foreign proteins enter the body.[11] Eosinophils can phagocytize, kill, and digest bacteria and yeast. Elevations of eosinophils counts are highly suggestive of parasitic infections.

Basophils are present in small numbers in the peripheral blood and are the most long-lasting granulocyte in blood with a circulating lifespan of approximately two weeks.[2] They contain heparin, histamine, and leukotriene B_4.[42,43] Many signs and symptoms of allergic responses can be attributed to specific mast cell and basophil products.[42] Basophils are probably involved in immediate hypersensitivity reactions (e.g., extrinsic, or allergic, and asthma) in addition to delayed hypersensitivity reactions. Basophils may be increased in chronic inflammation and in some types of leukemia.

Monocytes/Macrophages

Normal range: monocytes 2–8% or 0.02–0.08

Monocytes leave the circulation in 16–36 hours and enter the tissues where they mature into macrophages. *Macrophages* are present in lymph nodes, alveoli of the lungs, spleen, liver, and bone marrow, comprising the reticuloendothelial system.[44] Macrophages, both those circulating and those that have migrated out of the blood, participate in the removal of foreign substances from the body. In addition to attacking foreign cells, they are involved in the destruction of old erythrocytes, denatured plasma proteins, and plasma lipids. Tissue macrophages also salvage iron from the hemoglobin of old erythrocytes and return the iron to transferrin for delivery to the bone marrow. Under appropriate stimuli, monocytes/macrophages are transformed into antigen-presenting cells (APC, also termed *dendritic cells*). These transformed macrophages are an important component of both cell-mediated (T lymphocytes) and soluble (B lymphocyte) immune activity against antigens.[44]

Lymphocytes and Plasma Cells

Normal range: lymphocytes 20–40% or 0.2–0.4

Lymphocytes make up the second major group of leukocytes. They are characterized by a far less granular cytoplasm and relatively large, smooth nuclei. These cells give specificity and memory to the body's defense against foreign invaders.[45] There are three subgroups of lymphocytes:

1. T lymphocytes (T cells)
2. B lymphocytes (B cells)
3. Natural killer cells (NK cells)

Lymphocytes are not phagocytic, but the NK and T-cell subtypes are cytotoxic by virtue of complement activation and antibody-dependent cell-mediated cytotoxicity (ADCC). Morphologic differentiation of lymphocytes is difficult; visual inspection of a blood smear cannot uniformly distinguish between T, B, and NK cells. Fortunately, lymphocytes can be distinguished by the presence of CD lineage-specific membrane markers. Thus, mature T cells have CD3 and CD5, B cells have CD20, and NK cells have CD56 membrane markers.[43,46] Individual CD moieties may be surface proteins, enzymes, or adhesion molecules, to name a few. Labeled antibodies to specific CD molecules will identify the lineage of the lymphocyte, either in blood or in tissue.

Identification of the subtype of lymphocytes is not a routine clinical hematology test at present; they are reported simply as lymphocytes by automated counting instruments. However, in research applications and for the diagnosis and guidance of targeted treatment of leukemias and lymphomas, subtypes can both be counted and sorted by an automated process termed *fluorescence-activated cell sorting*. The WBC layer is separated by centrifugation and exposed to one or more CD-antibodies tagged with fluorescent dyes. The labeled cells are given an electrostatic charge and then flow individually past one or more lasers that induce the labeled cells to glow at wavelengths specific to the dye staining each cell. These fluorescing cells are counted and then can be sorted by electrostatic charges to push or pull the charged cell in the desired direction as they fall past the detector into collection vessels. This method is very general and can be used to count and sort virtually any cell that can be labeled with a fluorescent tag.[47] As discussed below, the identification of specific lymphocyte subtypes in this manner is increasingly used in determining the optimal treatment of various hematologic neoplasms.

With the help of T cells, B cells recognize foreign substances and are transformed into plasma cells, capable of producing antibodies (discussed later). **Table 16-6** lists the types of disorders in which lymphocytes are increased or decreased.

T Lymphocytes

T lymphocytes are responsible for cell-mediated immunity and are the predominant lymphocytes in circulation and in tissue. They require partial maturation in the embryonic thymus, hence, the name *T cell.* In addition to identifying infections, they oversee delayed hypersensitivity (seen with skin tests for TB, mumps, and *Candida*) and rejection of transplanted organs.[43] For a foreign antigen to be recognized by T cells, it must be "presented" by macrophages or dendritic cells on one of two complex, individualized molecules termed *major histocompatibility complexes* (MHC1 and MHC2).

T cells can be further divided into helper and cytotoxic (or suppressor) cells, which, respectively, express the CD4 and CD8 markers. CD4 helper cells are not cytotoxic, but on recognizing an antigen will activate and produce cytokines such as IL-2, which stimulate nearby immune cells including macrophages and CD8 T cells, B cells, and NK cells.

CD4 T-helper cells can again be divided into T_{H1} and T_{H2} subtypes. The T_{H1} subtype mediates the activation of macrophages and the delayed hypersensitivity response, while the T_{H2} subtype appears primarily responsible for B-cell activation. The cellular specificity of these subtypes appears to arise primarily from their distinct pattern of cytokine production.

The HIV virus binds specifically to the CD4 receptor but does not elicit the desired antiviral response in most patients. This infection leads to destruction of this subset of T cells and a reversal of the CD4/CD8 ratio (normally >1). The CD4 lymphocyte count and viral burden measured by viral RNA are inversely related and correlate with overall prognosis. Although the CD4 count remains a useful surrogate marker in monitoring the course and treatment of HIV-infected patients, viral loads are also increasingly measured. The lack of adequate numbers of active T-helper cells that activates other immune cells leads to an increased susceptibility to numerous opportunistic infections, cancer, and AIDS.[48,49] T cells are the primary mediator for host rejection of transplanted solid organs such as heart, lung, kidney, liver, and pancreas grafts. The perioperative and postoperative treatment of solid organ graft recipients is directed toward minimizing the antigraft T-cell response, while not ablating the T-cell population to the point of causing life-threatening infections. In practice, this is a narrow path plagued by viral and fungal infections that cause substantial morbidity and mortality in graft recipients.

Typically, T-cell populations in graft recipients are not measured, and drug titration is based on biopsies of the transplanted organ, drug concentrations of the immunosuppressants, and blood counts. Anti-T-cell treatments employed in transplant recipients include corticosteroids; Muromonab, anti-CD3 antibody directed against the CD3 marker found on T cells; antihuman lymphocyte immunoglobulin; and inhibitors of T-cell activation such as tacrolimus or mycophenolate. Because the immunoglobulins are typically obtained from nonhuman species, they can cause severe allergic reactions and are usually effective for only a short period.

TABLE 16-6. Quantitative Disorders of White Blood Cells[15,40,41,52,53]

WBC ABNORMALITY	TYPICAL THRESHOLD (cells/µL)	POSSIBLE CAUSES
Neutrophilia	>12,000	Acute bacterial infection Trauma Myocardial infarction Chronic bacterial infection Epinephrine, lithium, G-CSF, GM-CSF, glucocorticosteroids
Neutropenia	<1500	Radiation exposure Medications: Antineoplastic cytotoxic agents. Captopril Carbamazepine Cephalosporins Chloramphenicol Clozapine Diclofenac Ganciclovir Levamisole Methimazole Penicillins Phenothiazines Procainamide Propylthiouracil Ticlopidine Vancomycin Zidovudine Tricyclic antidepressants Sulfamethoxazole–trimethoprim Overwhelming acute bacterial infection Vitamin B_{12} or folate deficiency Salmonellosis Pertussis
Eosinophilia	>350	Allergic disorders/asthma Parasitic infections Leukemia Medications Angiotensin-converting enzyme inhibitors Antibiotics (or any allergic reaction to a drug)
Eosinopenia	<50	Acute infection
Basophilia	>300	Chronic inflammation Leukemia
Monocytosis	>800	Recovery state of acute bacterial infection Tuberculosis (disseminated) Endocarditis Protozoal or rickettsial infection Leukemia
Lymphocytosis	>4000	Infectious mononucleosis Viral infections (e.g., rubella, varicella, mumps, cytomegalovirus) Pertussis Tuberculosis Syphilis Lymphoma
Lymphopenia	<1000	HIV type 1 Radiation exposure Glucocorticosteroids Lymphoma (Hodgkin disease) Aplastic anemia

G-CSF = granulocyte-colony stimulating factor; GM-CSF = granulocyte-macrophage colony-stimulating factor; HIV = human immunodeficiency virus; WBC = white blood cell.

B Lymphocytes

B cells are named after similar avian lymphocytes that required maturation in an organ termed the *Bursa of Fabricius*. There is no equivalent organ in humans, and maturation of B lymphocytes occurs in the bone marrow. Quiescent, circulating B cells express one form of antibody, immunoglobulin M. When stimulated by activated T cells or antigen-presenting cells (APC), B cells are transformed into plasma cells that will produce one of five immunoglobulin types: IgA, IgD, IgE, IgG, or IgM.[43]

The two antibodies most commonly associated with the development of immunity to foreign proteins, viruses, and bacteria are IgM and IgG. IgE is associated with the development of allergic phenomena. IgA is secreted into the lumen of the GI tract and helps avoid sensitization to foodstuffs, and IgD is bound to the lymphocyte cell membrane.[43] Abnormal immunoglobulins can typically be detected using serum protein electrophoretic (SPEP) gels. Monoclonal hyperimmunoglobulinemias are identified by single peaks on SPEP gels and are typically associated with plasma (B) cell neoplasms. Polyclonal hyperimmunoglobulinemias can be associated with infections and inflammatory reactions.

Lymphopenia and hypogammaglobulinemia (a decrease in the total quantity of immunoglobulin) are seen as a consequence of steroid treatment, transplant rejection prophylaxis, and anticancer treatment, but they can also paradoxically arise from leukemias. In general, lymphopenia is more common in chemotherapy regimens that include high doses of glucocorticosteroids. Glucocorticosteroids bind to a receptor on lymphocytes and are lymphotoxic, even to the point of initiating cellular apoptosis.[46] Interestingly, although HIV-1 infections lead to lymphopenia, other viral infections (e.g., infectious mononucleosis, hepatitis, mumps, varicella, rubella, herpes simplex, herpes zoster, and influenza) often increase the number of circulating lymphocytes (lymphocytosis).[50,51] (**Minicase 5**.)

Natural Killer Cells

Natural killer cells (NK) are derived from T-cell lineage but are not as restricted in requiring MHC identification of the target

MINICASE 5

ANEMIA AND LYMPHOPENIA

Donna L. is a 55-year-old female with a history of rheumatoid arthritis and type II diabetes mellitus who presents to her physician for a routine physical examination. She is feeling well and has no complaints, other than the soreness routinely associated with the arthritis in her hands. She has normal vital signs and other than the stigmata of her moderate rheumatoid arthritis, she has a normal physical examination. She takes the following oral medications routinely:

Prednisone 5 mg once daily with dinner
Metformin 750 mg once daily with dinner
Methotrexate 10 mg weekly
Acetaminophen 650 mg q 6 hr PRN for hand pain

The physician draws a comprehensive metabolic panel and a CBC with differential and platelet count. The results of the CBC with differential and platelet count are below:

TEST NAME	RESULT	REFERENCE RANGE
RBC	4×10^6 cells/μL	$4.1–5.1 \times 10^6$ cells/μL for females
WBC	9.6×10^3 cells/μL	$4.4–11.3 \times 10^3$ cells/μL
Hgb	13.3 g/dL	12.3–15.3 g/dL for females
Hct	37.9%	36–45% for females
MCV	105.5 fL/cell	80–96 fL/cell
MCH	39.2 pg/cell	27–33 pg/cell
RDW	15%	11.5–14.5%
Platelet	304,000 cells/μL	150,000–450,000 cells/count μL
Neutrophils	76%	45–73%
Bands	5%	3–5%
Monocytes	7%	2–8%
Eosinophils	2%	0–4%
Basophils	1%	0–1%
Lymphocytes	9%	20–40%

QUESTION: What abnormalities are present, and what is their cause and resolution?

DISCUSSION: This patient has somewhat low RBC count and hemoglobin as well as elevated MCV and MCH, indicating a macrocytic anemia. She also is demonstrating a high leukocyte count with an elevated neutrophils and decreased lymphocyte count. She is not showing signs of infection. The macrocytic anemia could be due to hypovitaminosis B_{12} or folate deficiency. The chronic dosing with methotrexate, an antifolate, is the likely cause. The differential diagnosis can be made by obtaining blood assays for vitamin B_{12} and folate. Many clinicians will prescribe 5 mg oral folate daily except for methotrexate dosing days, and this would be an appropriate recommendation for this patient as well.

The lymphopenia and neutrophilia are likely caused by the daily prednisone doses. Glucocorticoids are known to cause demargination of neutrophils from the vascular endothelium, leading to a relative neutrophilia. Glucocorticoids are also lymphotoxic, typified in their use the treatment of lymphocytic malignancies. No treatment is indicated in this patient, but monitoring for opportunistic infections such as candidiasis needs to be ongoing. The steroid-induced changes in lymphocyte and neutrophil counts are expected to return to normal after the cessation of the steroid dosing.

cell. NK cells are thought to be particularly important for cytotoxic effects on virally infected cells and cancer cells.

Leukocyte Disorders

Patients can suffer from three major classes of *leukocyte disorders:* functional, quantitative, and myeloproliferative. *Functional* disorders involve defects in recognition, metabolism, cytotoxic effects, signaling, and other related activities. Routine laboratory values are not intended to evaluate these abnormalities and will not be discussed further here.

Quantitative disorders involve too few or too many leukocytes. Possible causes are listed in Table 16-6. Neutropenia is usually considered to exist when the neutrophil count is <1500 or 1000 cells/μL.[38,52] When the neutrophil count is <500 cells/μL, normal defense mechanisms are greatly impaired, and the patient is at increased risk of spontaneous bacterial and fungal infections. A neutrophil count <100/μL is termed *absolute neutropenia* or *agranulocytosis.* This is usually encountered after chemotherapy is administered, especially following regimens intended to ablate the bone marrow in preparation for a stem cell transplant. An infection is probable if agranulocytosis is prolonged, so patients at risk are often given prophylactic antibiotics. When infections do occur in such patients, they can be very difficult to successfully treat—even with normally effective antibiotics—because the number and phagocytic activity of the neutrophils are impaired.

Agranulocytosis may be caused by aplastic anemias that reflect inadequate myelopoiesis. Aplastic anemias (inadequate production of blood cells by the bone marrow) have multiple causes including drug, toxin, or radiation exposure; congenital defect; or age-related fatty or fibrotic bone marrow replacement. The word *anemia* in this term is misleading because production of other blood cell types can also be decreased resulting in pancytopenia.

Myelodysplastic anemias are characterized by abnormal maturation of RBCs and WBCs. These are typically classified by the French-American-British (FAB) or the more recent World Health Organization (WHO) system based on the marrow morphology identified from a bone marrow aspirate. The usual treatment course is supportive care (i.e., transfusions or stem cell transplant in patients for whom this is feasible).[54]

Neutrophilia (increased circulating neutrophils) is caused by both increased release from the bone marrow and a shift of marginated cells into the circulation. This rapid rise in the number of circulating cells can be caused by acute infections, trauma, or administration of epinephrine or corticosteroids. Prolonged neutrophilia may be due to sustained overproduction caused by ongoing bacterial infections or tissue damage (e.g., cell death, infarction).

Leukemias

Neoplasms of the bone marrow cells usually involve a leukocyte line and are termed *leukemias*. Leukemias are broadly classified as being acute or chronic, and leukemias are either of myeloblastic (granulocytic lineage) or lymphoblastic (lymphocytic) lineage.[55] The clinical course and biology of various leukemias varies. Almost all leukemias fall within one of the four categories:

1. Acute myelogenous (AML)
2. Acute lymphoblastic (ALL)
3. Chronic myelogenous (CML)
4. Chronic lymphocytic (CLL)

Although the clinical course will vary among these neoplasms, a common denominator is the proliferation of the neoplastic cell line and displacement of normal hematopoiesis. The neoplastic cells may arise from cells of varying levels of differentiation of either a granulocytic or lymphocytic lineage. Morphology and CD membrane markers will vary among individuals but be fairly uniform throughout the disease course in a given patient. The morphology and CD membrane markers of cells obtained from the diagnostic bone marrow aspirate and flow cytometry respectively are used to assign an FAB classification of M0 through M7 to subtype AML or to diagnose ALL. Other morphologic features and surface marker combinations are used to characterize the other leukemias.

Multiple (plasma cell) myeloma is notable in that it is a plasma cell neoplasm of the bone marrow. The monoclonal neoplastic plasma cells produce a single immunoglobulin isotype (IgG, IgA, light chain only, IgD, IgE, or rarely IgM). This single, monoclonal protein is referred to as the *M-protein*. The M-protein is usually identified using serum protein electrophoresis. The specific immunoglobulin type can be defined with a subsequent step of serum immunofixation with protein-specific antibody (e.g., anti-IgG). Other laboratory findings associated with multiple myeloma include Bence Jones protein (light chain) in urine, hypercalcemia, increased ESR, normochromic, normocytic anemia, and coagulopathy.[56]

Chronic *myeloproliferative* disorders involve an abnormal proliferation of more mature bone marrow cells. Excessive or uncontrolled proliferation of all cell lines leads to polycythemia vera, a malignancy when erythrocyte overproduction is the most prominent abnormality. CML is characterized by a chromosomal translocation [t(9:22), “Philadelphia chromosome”] that creates a fusion product (BCR/ABL) resulting in autonomous tyrosine kinase activity, a growth signaling enzyme. Some patients without the Philadelphia chromosome have been thought to have CML in the past. However, new techniques suggest that the translocation is fundamental to the diagnosis of CML, and that in its absence, these individuals are more likely to have some other myeloproliferative disorder.[57] Patients with CLL present with increased numbers of circulating mature B lymphocytes, which are monoclonal.

Patients with chronic leukemias may live for several years with minimal treatment because of the indolent nature of the disease. At some point the patient will typically develop a transformation of their disease into a life-threatening accelerated phase or blast crisis. Fortunately with the development of tyrosine kinase inhibitors and other targeted medications, this fatal complication can now often be substantially delayed. Although the chronic leukemias are less aggressive than the acute leukemias, they are less curable with chemotherapy, and stem cell transplantation is appropriate in selected patients.

Lymphomas. A lymphoma is a neoplasm of lymphocytic lineage, which typically predominates in lymph nodes forming tissue masses rather than being primarily located in the bone marrow. The lymphomas are classified into two main groups: (1) non-Hodgkin lymphoma (NHL), and (2) Hodgkin lymphoma. The pattern of tissue involvement— termed either *diffuse* or *follicular* (nodular)—and the cytology of the neoplastic lymphoid cells (primarily the size and appearance of the cell nucleus) are used to morphologically subclassify non-Hodgkin lymphoma.[58] The WHO classification of NHL also uses CD surface markers, cytogenetics, and molecular studies to further define subcategories of NHL. Non-Hodgkin lymphomas can also be practically divided into aggressive and indolent forms. The aggressive lymphomas grow and spread quickly but are generally more likely to be eradicated with current, intensive chemotherapy. In contrast, the slower-growing, indolent lymphomas are not as responsive and are more difficult to cure, but these often have a long disease course. Hodgkin lymphoma is generally a more treatable lymphoma. The neoplastic cellular element is termed the *Reed-Sternberg cell*. This is a very large cell with a lobulated nucleus and prominent nucleoli. It is typically surrounded by a non-neoplastic population of lymphocytes, eosinophils, neutrophils, plasma cells, and macrophages.

Lymphomas predictably involve T-lymphocyte or B-lymphocyte precursors, and many express CD marker characteristics of mature lymphocytes. Identification of the CD20 marker on B-cell lymphomas provides an opportunity to treat these patients with recombinant antibodies specific to this surface marker. Differentiation between a T-cell leukemia and a peripheral T-cell lymphoma will likely require the identification of CD phenotypes.

Leukocyte Phenotype-Guided Drug Therapy

Translational research is increasingly successful in identifying both gross and subtle differences between normal and cancerous cells. These differences can then be used to develop new diagnostic and therapeutic tools for the treatment of patients with both hematogenous and solid tumors. For example, patients with leukemias expressing the BCR/ABL fusion protein are likely to be treated with tyrosine kinase inhibitors such as imatinib, dasatinib, or nilotinib.[59] Identifying the presence of abnormal surface or intracellular markers increases the likelihood that the use of a drug that acts specifically on such cells will be effective. Such drugs could be administered blindly to all patients without identifying the malignant phenotype, but the likelihood of response would be less, and the cost of drug administered to patients with little or no likelihood of response prohibitively high.

Another example of targeted treatment of lymphocytes is the use of antibodies such as rituximab to CD20 to treat patients identified as having CD20+ lymphomas. The Fab portion of the antibody binds to the CD20 epitope on the lymphocytes, and the Fc portion of the antibody activates T-cell-mediated cytotoxicity to cause destruction of the bound cell.[60] The same CD20 epitope on the lymphomas is being used for the targeted immune and radioactive cytotoxicity of ibritumomab treatment.[61] This treatment combines rituximab with ibritumomab, another CD20-directed antibody linked to indium-111 or to yttrium-90. Ibritumomab-^{111}In is administered first, and the emitted γ rays are imaged to assess appropriate distribution of the CD20 antibody. If acceptable, subsequent doses of

TABLE 16-7. Cell Types, Associated Target or CD Epitopes, and FDA-Approved Targeted Therapies

CELL TYPE	ASSOCIATED TARGET OR CD EPITOPE	FDA-APPROVED TARGETED THERAPIES
B-cell non-Hodgkin lymphoma	CD-20	Rituximab Ofatumumab ^{90}Y Ibritumomab ^{131}I Tositumomab
Cutaneous T-cell lymphoma	CD25 component of IL-2 receptor	Denileukin diftitox
Hodgkin lymphoma Anaplastic large cell lymphoma	CD30	Brentuximab vedotin
Multiple myeloma	CD38 Signaling lymphocytic activation molecule family member 7 (SLAMF7, CD319)	Daratumumab Elotuzumab
Peripheral T-cell lymphoma	CD19 (B-cell) and CD3 (T-cell) coligand	Blinatumomab (CML Ph-)
Chronic lymphocytic leukemia	CD20 CD52 (Campath)	Obinutuzumab Alemtuzumab
Chronic myelogenous leukemia	Philadelphia chromosome (BCR-Abl)	Bosutinib Imatinib mesylate Dasatinib Nilotinib Ponatinib

CD = Cluster of Differentiation; FDA = U.S. Food and Drug Administration.

ibritumomab-^{90}Y are administered to irradiate the CD20+ lymphoma cells with β particles.

Denileukin diftitox is another novel cytotoxic drug that is presently indicated for CD25+ cutaneous T-cell lymphomas (mycosis fungoides and Sézary syndrome), but that is also being explored for the treatment of other T-cell mediated disorders such as steroid-refractory graft-verus-host disease and systemic T-cell lymphomas.[62] Denileukin diftitox is a fusion protein composed of human IL-2 fused with diphtheria toxin. The IL-2 moiety of the drug preferentially binds to cells that express the IL-2 receptor (e.g., T cells containing CD25/CD122/CD152). Once the IL-2 receptor and bound drug are internalized and the protein cleaved, the diphtheria toxin is activated and causes cell death due to protein inhibition. Other targeted immunotherapies use monoclonal antibodies to identify the CD on malignant cells; ADCC by macrophages; and activated T, natural killer, and dendritic cells. **Table 16-7** shows CD epitopes associated with various hematologic or lymphatic malignancies and the drugs targeting these cells that were FDA-approved at the time of this edition. Additional targeted therapies are available for hematologic and solid tumor malignancies but are not included in this table.

SUMMARY

This chapter presents a brief characterization of the lineage and function of RBCs and WBCs. Normal laboratory values have been presented, but it is important to realize that normal ranges will vary slightly depending on the laboratory conducting the analysis and the population being studied.

In hematology, as in most medical sciences, it is important to consider the background and context of the tests used. For example, the Wintrobe RBC indices were characterized at a time when the iron-transporting proteins and vitamin needs of erythropoiesis were unknown. In most cases, abnormalities of the indices reflect a long-term inadequacy of iron, folate, or vitamin B_{12}. Biochemical markers, such as circulating ferritin, transferrin receptors, folate, homocysteine, and methylmalonate, are likely to be of increasing importance in the early detection of such deficiencies.

Similarly, the definition of lineage-specific markers (CD phenotypes) on leukocytes has revolutionized our ability to diagnose and treat leukemias, lymphomas, and other malignancies. General diagnoses such as AML will likely continue to be used, but increasingly specific characterization of the surface markers, biochemistry, and genetics of cells will provide new opportunities to more effectively treat such diseases.

Although there is increasing precision and sophistication in identifying molecular changes associated with hematologic pathologies, the importance of understanding the fundamentals of clinical hematology cannot be discounted. Infections and chronic leukemias will continue to be diagnosed and monitored from an elevated WBC count, and anemias will be identified and treated through routine blood examinations. Old and new technologies will increasingly complement one another in clinical hematology.

LEARNING POINTS

1. ***How do iron deficiency and nutrient deficiency (folate and vitamin B_{12}) differ in their presentation in a hemogram?***

   ANSWER: As expressed by the term *anemia,* in each of these circumstances the total RBC (erythrocyte) count will be low, as will the Hgb and Hct. Iron deficiency is characterized by small (microcytic, low MCV) and pale (hypochromic) erythrocytes. In contrast, both folate and vitamin B_{12} deficiency classically present with larger (macrocytic, elevated MCV) erythrocytes. Another difference often noted in the hemogram is an elevated reticulocyte count in patients with iron deficiency after treatment with supplemental iron.

2. ***What are the roles of transferrin, ferritin, and TIBC, and how are laboratory values for these substances interpreted?***

   ANSWER: Transferrin is a plasma protein with high avidity to highly reactive metal ions such as iron and chromium. Its primary role is to transport iron to the bone marrow for erythrocyte synthesis, while in the process protecting intervening tissue from the reactivity of the metal ion. Ferritin is another minor iron plasma transport protein but differs from transferrin in that it enters cells of the reticuloendothelial system, where it serves its greater role as the storage form of iron. Ferritin protein not bound to iron is termed *apoferritin.* Most of the iron-binding protein in the plasma is transferrin, and the serum TIBC is an indirect measure of the transferrin concentration. When iron stores are low (iron deficiency), the liver synthesizes more transferrin. Thus, the residual, unbound capacity of the transferrin (and thus TIBC) will be increased. In anemia of chronic disease, the plasma iron and transferrin concentrations are both low, so although the transferrin saturation may be decreased, it will often be within the normal range. Liver disease or malnutrition can also slow the production of transferrin, which may complicate the interpretation of the TIBC.

3. ***What are typical reasons why WBC counts are elevated, and how can the differential cell count help clarify the causality?***

   ANSWER: A sustained elevation of the WBC count is typically due to infections or leukemias. Infections, epinephrine, and exercise cause a demargination of neutrophils from the endothelium, causing a transient, increased percentage of neutrophils but a normal absolute lymphocyte count. In contrast, corticosteroids also cause neutrophil demargination but are also lymphotoxic, so the absolute lymphocyte count will decrease. Bacterial infections are associated with an increase in the percentage and absolute number of neutrophils and to the release of premature neutrophils (band cells) from the bone marrow. Very high or low WBC counts, or abnormal differential count percentages and unusual cell morphology increase the suspicion of leukemia.

4. ***What are common, unintended drug-induced alterations in RBC and WBC counts and function?***

   ANSWER: RBC counts can be reduced by NSAID-induced gastrointestinal bleeding or by hemolytic anemia in patients with G6PD deficiency treated with various oxidizing drugs. Both RBC and WBC counts are commonly decreased following cytotoxic chemotherapy, but the impact on WBCs is greater, especially for neutrophils, because of their faster turnover and shorter lifespan. Macrocytic, hypochromic anemia can be caused by treatment with antifolates such as methotrexate or chronic treatment with antibiotics inhibiting DNA synthesis. Glucocorticosteroids are lymphotoxic and will decrease the lymphocyte count but will also lead to a higher apparent neutrophil count due to their drug-induced demargination from the endothelium.

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17

HEMATOLOGY: BLOOD COAGULATION TESTS

Lea E. Dela Peña

OBJECTIVES

After completing this chapter, the reader should be able to

- Describe the role of platelets, the coagulation cascade, and fibrinolytic system in normal hemostasis
- List the laboratory tests used to assess platelets and discuss factors that may influence their results
- List the laboratory tests used to assess coagulation and explain their use in evaluating anticoagulant therapy
- List the laboratory tests used to assess clot degradation and disseminated intravascular coagulation and discuss their limitations
- Interpret results and suggest follow-up action given results of laboratory tests used for evaluating coagulation and anticoagulant therapy in a case description
- Discuss the availability and use of point-of-care testing devices specifically for platelet and coagulation tests

Normal hemostasis involves a complex interaction among the vascular subendothelium, platelets, coagulation factors, and proteins that promote clot formation, clot degradation and inhibitors of these substances. Disruption in normal hemostasis can result in bleeding or excessive clotting. Bleeding can be caused by trauma or damage to vessels, acquired or inherited deficiencies of coagulation factors, or physiological disorders of platelets, whereas excessive clotting can result from abnormalities of the vascular endothelium, alterations in blood flow, or deficiencies in clotting inhibitors.

Clinicians must monitor the hemostasis process in individual patients to ensure their safety from an imbalance in this complex system. For example, practitioners routinely order platelet tests in patients on certain antineoplastic medications to assess for thrombocytopenia. Likewise, clinicians closely monitor coagulation tests for patients receiving anticoagulants to prevent thromboembolic or hemorrhagic complications. Overall, the hemostatic process is intricate and requires a clinician knowledgeable in its dynamics for quality assessment.

This chapter reviews normal coagulation physiology, common tests used to assess coagulation and hypercoagulable states, and factors that alter coagulation tests.

PHYSIOLOGICAL PROCESS OF HEMOSTASIS

Normal *hemostasis* involves the complex relationship among participants that promote clot formation (platelets and the coagulation cascade), inhibit coagulation, and dissolve the formed clot. Each phase of the process is briefly reviewed.

Clot Formation

Numerous mechanisms promote and limit coagulation. Factors that promote coagulation include malignancy, estrogen therapy, pregnancy, obesity, immobilization, damage to the blood vessel wall, and causes of low blood flow or venous stasis. Normal blood flow dilutes activated clotting factors and results in their degradation in various tissues (e.g., liver) and by proteases. However, when low flow or venous stasis is present, activated clotting factors may not be readily cleared.

Platelets

Platelets are non-nucleated, disk-shaped structures, 1–5 microns in diameter, which are formed in the extravascular spaces of bone marrow from megakaryocytes. Megakaryocyte production and maturation are promoted by the hormone thrombopoietin, which is synthesized in the bone marrow and liver. Two thirds of the platelets are found in the circulation and one third in the spleen; however, in splenectomized patients nearly 100% are in the circulation.

The average human adult makes approximately 100 billion platelets per day, with the average platelet circulating for 7–10 days. On aging, platelets are destroyed by the spleen, liver, and bone marrow. Throughout their lifespan, platelet function is affected by numerous factors such as medications, vitamins, foods, spices,

Note: The contribution of material written by James B. Groce III, Julie B. Lemus, and Sheila M. Allen in previous editions of this book is gratefully acknowledged.

and systemic conditions, including chronic renal disease and hematological disorders (e.g., myeloproliferative and lymphoproliferative diseases, dysproteinemias, and the presence of antiplatelet antibodies).

The primary function of platelets is to regulate hemostasis, but platelets also play a prominent role in the pathological formation of arterial thrombi. Three processes (platelet adhesion, activation, and aggregation) are essential for arterial thrombus formation. The surface of normal blood vessels inhibits platelet function, thereby preventing thrombosis; however, endothelial injury to the vasculature, caused by flow abnormalities, trauma, or the rupture of atherosclerotic plaque in the vessel wall, starts the process of platelet plug formation. Subendothelial structures—such as collagen, basement membrane, and fibronectin—then become exposed (**Figure 17-1**), which can result in platelet adhesion. Platelet adhesion is enhanced by substances such as epinephrine, thrombin, adenosine diphosphate (ADP), serotonin, collagen, and von Willebrand factor (vWF).[1] Circulating vWF acts as a binding ligand between the subendothelium and glycoprotein Ib receptors on the platelet surface.

Once adhesion occurs, platelets change shape and activation occurs. Activated platelets release their contents—including nucleotides, adhesive proteins, growth factors, and procoagulants—which promotes platelet aggregation and completes the formation of the hemostatic plug.[2] This process is mediated by glycoprotein IIb/IIIa receptors on the platelet surface with fibrinogen acting as the primary binding ligand bridging between platelets. Platelets have numerous Gp IIb/IIIa binding sites, which are an attractive option for antiplatelet drug therapy.[1] However, the platelet plug is not stable and can be dislodged. To form a more permanent hemostatic plug, the clotting system must be stimulated. By releasing PF3, platelets initiate the clotting cascade and concentrate activated clotting factors at the site of vascular (endothelial) injury.

Prostaglandins (PGs) play an important role in platelet function. **Figure 17-2** displays a simplified version of the complex arachidonic acid pathways that occur in platelets and on the vascular endothelium. Thromboxane A_2, a potent stimulator of platelet aggregation and vasoconstriction, is formed in platelets. In contrast, prostacyclin (PG2), produced by endothelial cells lining the vessel luminal surface, is a potent inhibitor of platelet aggregation and a potent vasodilator that limits excessive platelet aggregation.

Cyclooxygenase and PG2 are clinically important. An aspirin dose of 50–81 mg/day acetylates and irreversibly inhibits cyclooxygenase in the platelet. Platelets are rendered incapable of converting arachidonic acid to PGs. This effect of low-dose aspirin lasts for the lifespan of the exposed platelets (up to 12 days). Vascular endothelial cells also contain cyclooxygenase, which converts arachidonic acid to PG2. Aspirin in high doses (3000–5000 mg) inhibits the production of PG2.[3] However, because the vascular endothelium can regenerate PG2, aspirin's effect is much shorter here than on platelets. Thus, aspirin's effect at high doses may both inhibit platelet aggregation and block the aggregation inhibitor PG2. This phenomenon is the rationale for using low doses of aspirin 75–162 mg/day to help prevent myocardial infarction.[4]

In summary, a complex interaction between the platelet and blood vessel wall maintains hemostasis. Once platelet adhesion occurs, the clotting cascade may become activated. After thrombin and fibrin are generated, the platelet plug becomes stabilized with insoluble fibrin at the site of vascular injury.

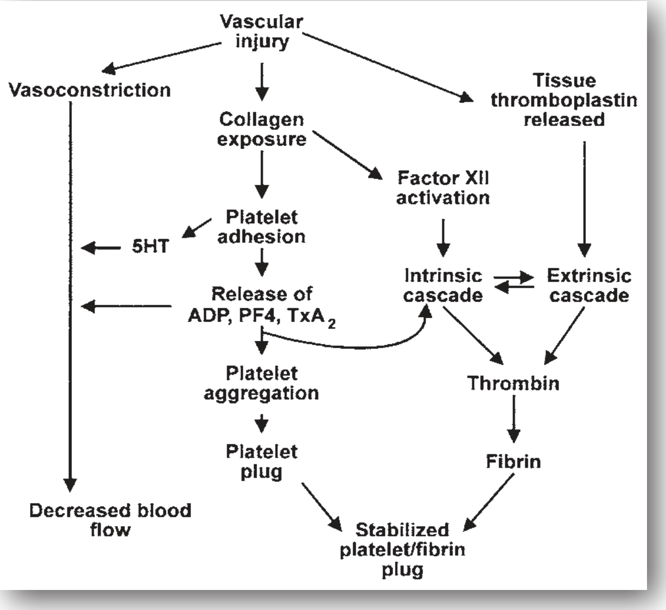
Vascular injury
Vasoconstriction
Collagen exposure
Tissue thromboplastin released
Factor XII activation
5HT
Platelet adhesion
Intrinsic cascade
Extrinsic cascade
Release of ADP, PF4, TxA2
Platelet aggregation
Thrombin
Platelet plug
Fibrin
Decreased blood flow
Stabilized platelet/fibrin plug

FIGURE 17-1. Relationship between platelets and the clotting cascade in the generation of a stabilized fibrin clot. 5HT = serotonin; ADP = adenosine diphosphate; PF4 = platelet factor 4; TxA_2 = thromboxane A_2.

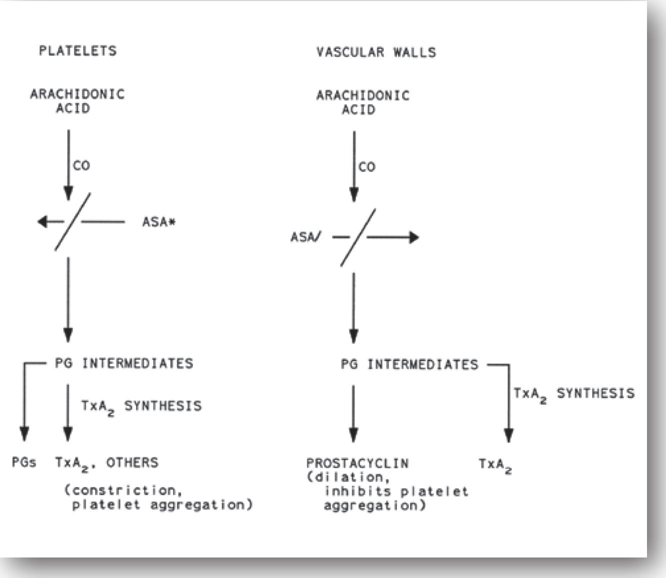
PLATELETS
VASCULAR WALLS
ARACHIDONIC ACID
ARACHIDONIC ACID
CO
CO
ASA*
ASA/
PG INTERMEDIATES
PG INTERMEDIATES
TxA2 SYNTHESIS
TxA2 SYNTHESIS
PGs
TxA2, OTHERS
(constriction, platelet aggregation)
PROSTACYCLIN
(dilation, inhibits platelet aggregation)
TxA2

FIGURE 17-2. Formation of thromboxane A_2 (TxA_2), prostaglandins (PGs), and prostacyclin in platelets and vascular endothelial cells. CO = cyclooxygenase; ASA* = low-dose, irreversible, inactivation of platelet cyclooxygenase; ASA/ = high-dose inactivation of platelet cyclooxygenase.

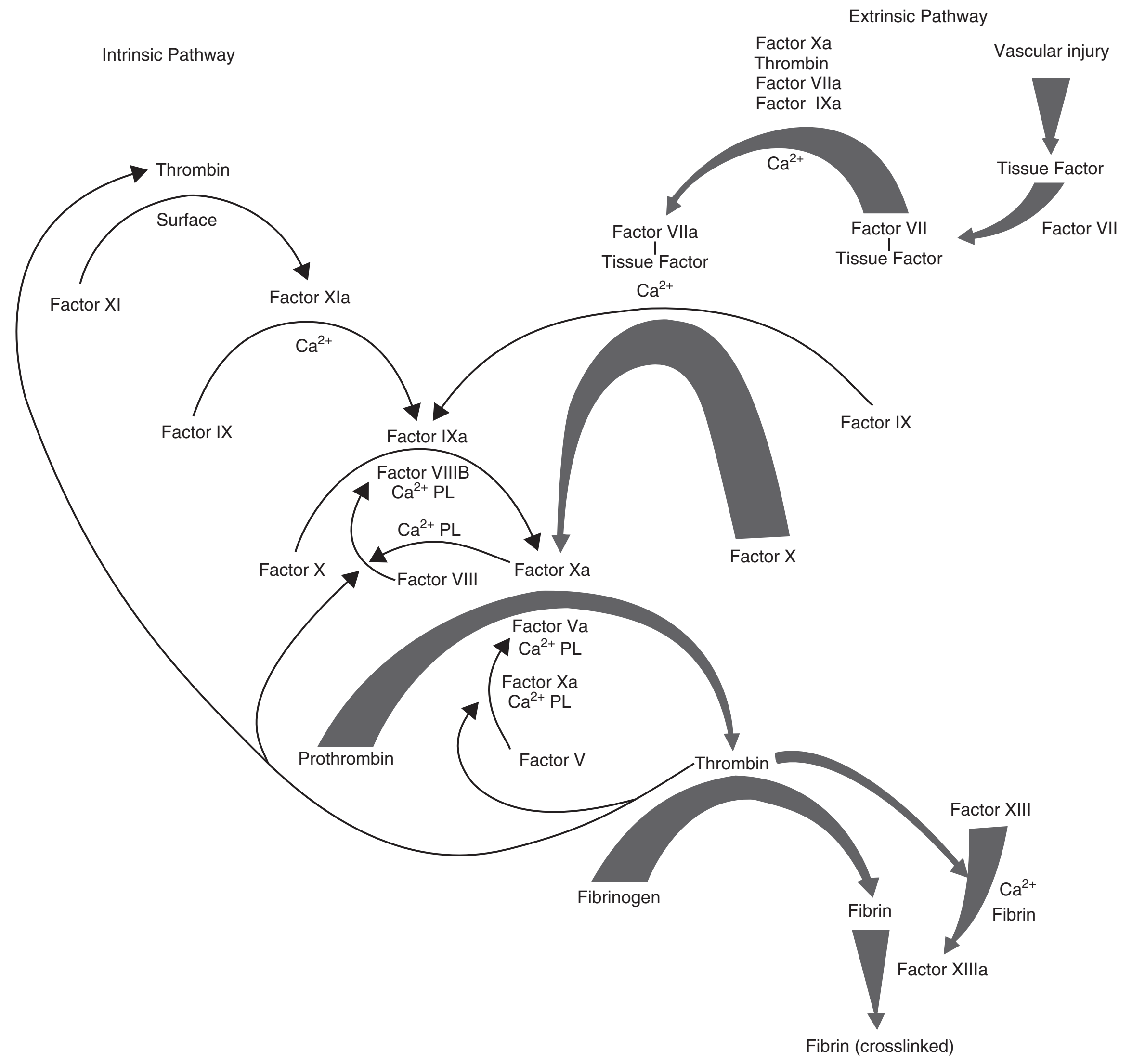
Extrinsic Pathway
Intrinsic Pathway
Factor Xa
Thrombin
Factor VIIa
Factor IXa
Vascular injury
Ca^{2+}
Tissue Factor
Thrombin
Surface
Factor VIIa
Tissue Factor
Factor VII
Tissue Factor
Factor VII
Ca^{2+}
Factor XI
Factor XIa
Ca^{2+}
Factor IX
Factor IXa
Factor IX
Factor VIIIB
Ca^{2+} PL
Ca^{2+} PL
Factor X
Factor VIII
Factor Xa
Factor X
Factor Va
Ca^{2+} PL
Factor Xa
Ca^{2+} PL
Prothrombin
Factor V
Thrombin
Factor XIII
Ca^{2+}
Fibrin
Fibrinogen
Fibrin
Factor XIIIa
Fibrin (crosslinked)

FIGURE 17-3. Coagulation cascade. a = activated factor; Ca = calcium; PL = phospholipid. *Source:* Adapted with permission from Davie EW, Fujikawa K, Kisiel W. The coagulation cascade; initiation, maintenance, and regulation. *Biochemistry.* 1991; 30;10363-70. Copyright© 1991. The American Chemical Society.

Coagulation Cascade

The ultimate goal of the *coagulation cascade* (**Figure 17-3**) is to generate fibrin from thrombin. Fibrin forms an insoluble mesh surrounding the platelet plug. Platelets concentrate activated clotting factors at the site of vascular injury.

The nomenclature and half-lives for the coagulation proteins are shown in **Table 17-1.** The coagulation cascade is typically divided into the intrinsic, extrinsic, and common pathways. The intrinsic and extrinsic pathways provide different routes to generate factor X, while the common pathway results in thrombin formation. Coagulation is initiated by vascular injury or damage that exposes blood to tissue factor (TF), which then binds to factor VII at the start of the extrinsic pathway. The binding of TF to factor VII activates the latter to VIIa. The complex formed by TF and factor VIIa can then activate factor X to Xa at the start of the common pathway. Alternatively, the TF-factor VIIa complex can first convert factor IX to factor IXa, with factor VIIIa as a cofactor, which is part of the intrinsic pathway. Factor IXa can then activate factor X into Xa; thus, both the intrinsic and extrinsic pathways activate factor X in the final common pathway. Factor Xa with factor Va as a cofactor activates prothrombin (factor II) into thrombin (factor IIa). In the clotting cascade, thrombin not only converts fibrinogen into fibrin, but it can also convert factor XIII to factor XIIIa, which stabilizes the fibrin clot. In addition to the direct effects and feedback mechanisms of thrombin shown in Figure 17-3, thrombin also stimulates platelet aggregation and activates the fibrinolytic system.

Additional factors within the pathway. Factors such as calcium and vitamin K play an intricate role within the various pathways in the coagulation cascade. Calcium is essential for the platelet surface binding of several factors within the pathway. Vitamin K facilitates the calcium binding function of factors II, VII, IX, and X via carboxylation. These processes are critical in activating proteins within the pathway.

TABLE 17-1. Characteristics of Coagulation Factor

FACTOR	NAMES	APPROXIMATE HALF-LIFE (HR)
I	Fibrinogen	100–150
II	Prothrombin	50–80
III	Thromboplastin, tissue extract	
IV	Calcium	
V	Proaccelerin, labile factor, accelerator globulin	12–36
VI	Originally referred to as accelerin, but now recognized as activated factor V; this numeral is no longer used	n/a
VII	Proconvertin, serum prothrombin conversion accelerator, stable factor, autoprothrombin I	4–66
VIII	Antihemophilic factor, antihemophilic globulin, platelet cofactor I, antihemophilic factor A	12–15
IX	Plasma thromboplastin component, Christmas factor, antihemophilic factor B, platelet cofactor II, autoprothrombin II	18–30
X	Stuart-Prower factor	25–60
XI	Plasmin thromboplastin antecedent	40–80
XII	Hageman factor	50–70
XIII	Fibrin stabilizing factor, Laki-Lorand factor, and fibrinase	150

n/a = not applicable.
Source: See reference 5.

TABLE 17-2. Mechanism of Action of Antithrombotic and Anticoagulant Medications

DRUG CLASS	SPECIFIC MEDICATIONS	MECHANISM OF ACTION
Platelet inhibitors	Aspirin	Irreversibly inhibits cyclooxygenase-1, which prevents conversion of arachidonic acid to thromboxane A_2
	Clopidogrel (Plavix) Prasugrel (Effient) Ticagrelor (Brilinta)	Irreversibly binds to $P2Y_{12}$ receptors on platelets
Oral anticoagulants	Warfarin (Coumadin, Jantoven)	Inhibits vitamin K dependent clotting factors (II, VII, IX, X) as well as protein C and protein S
	Rivaroxaban (Xarelto) Apixaban (Eliquis) Edoxaban (Savaysa)	Inhibits factor Xa
	Dabigatran (Pradaxa)	Direct thrombin inhibitor
Parenteral anticoagulants	Unfractionated heparin	Inhibits factor IIa
	Low molecular weight heparin: Enoxaparin (Lovenox) Dalteparin (Fragmin)	Inhibits factors IIa and Xa
	Fondaparinux (Arixtra)	Inhibits factor Xa
	Bivalirudin (Angiomax) Argatroban Desirudin (Iprivask)	Direct thrombin inhibitor

Inhibition of Coagulation

Mechanisms that limit *coagulation* include the natural inhibitors such as antithrombin (AT) and the vitamin K dependent proteins C and S, tissue factor pathway inhibitor (TFPI), and the fibrinolytic system. Endothelial cells produce several substances that have antithrombotic and anticoagulant effects, which may also activate the fibrinolytic system.[1] Several medications also can inhibit coagulation by acting on (1) platelets, such as aspirin, clopidogrel, prasugrel, and ticagrelor, or (2) one or more clotting factors, such as warfarin; low molecular weight heparins (LMWHs); unfractionated heparin (UFH); direct oral anticoagulants (DOACs) including rivaroxaban, apixaban, edoxaban, and dabigatran; fondaparinux; and direct thrombin inhibitors (DTIs). **Table 17-2** lists the mechanism of action of these classes of drugs and provides specific examples.

High concentrations of thrombin, in conjunction with thrombomodulin, activate protein C, which can then inactivate cofactors Va and VIIIa. Thus, there is a negative feedback mechanism that will block further thrombin generation and subsequent steps in the coagulation cascade. Protein S is another of the body's natural anticoagulants and serves as a cofactor for protein C. AT inactivates thrombin as well as factors IX, X, and XI, and this process can be hastened by heparin. Heparin and AT combine one-to-one, and the complex neutralizes the activated clotting factors and inhibits the coagulation cascade. Deficiencies in these natural inhibitors can result in increased generation of thrombin, which can lead to recurrent thromboembolic events often starting at a young age. TFPI impedes the binding of TF to factor VII, essentially inhibiting the extrinsic pathway (Figure 17-3). UFH and LMWHs can release TFPI from endothelial cells and from platelets.[1] The complex mechanisms that limit thrombus formation are shown in **Figure 17-4.**

Clot Degradation

Fibrinolysis is the mechanism by which formed thrombi are lysed to prevent excessive clot formation and vascular occlusion. As discussed previously, fibrin is formed in the final common pathway of the clotting cascade. Tissue plasminogen activator (tPA) and urokinase plasminogen activator activate plasminogen, which generates plasmin. Plasmin is the enzyme that eventually breaks down fibrin into fibrin degradation products (FDPs). Medications can either activate (e.g., alteplase, reteplase, and tenecteplase) or inhibit (e.g., tranexamic acid and aminocaproic acid) fibrinolysis.

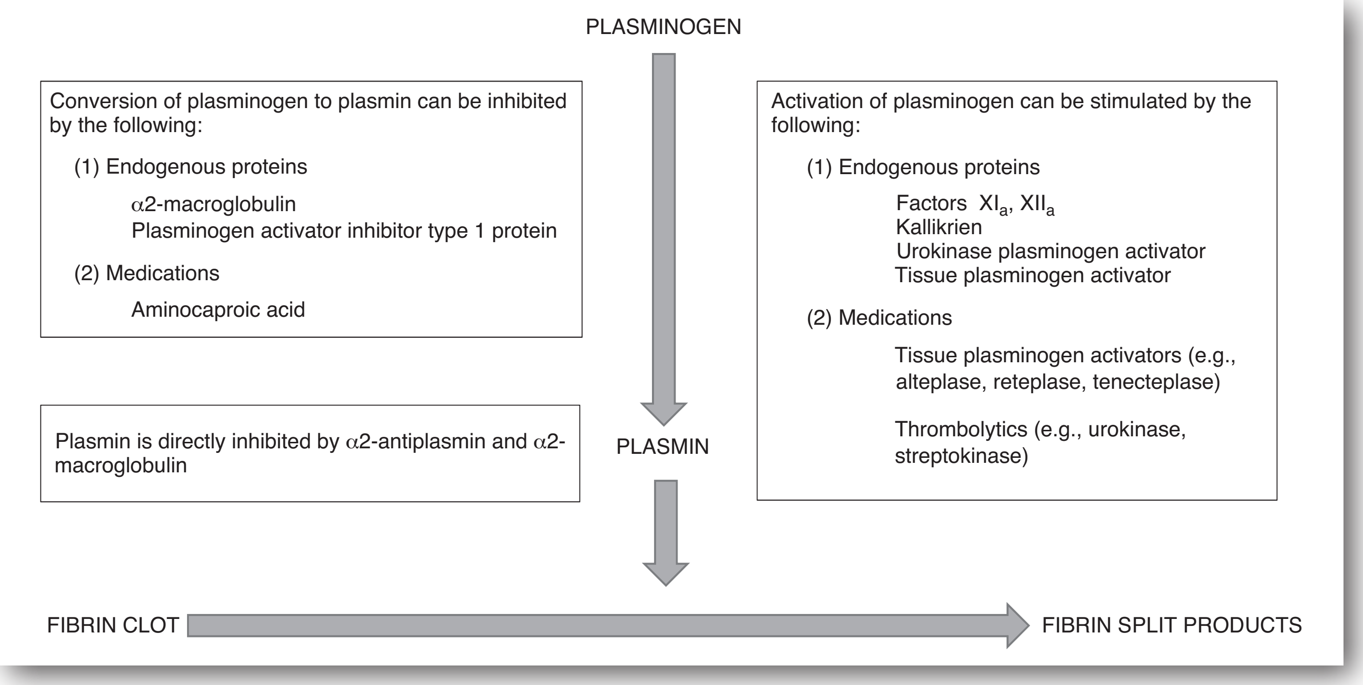
PLASMINOGEN
Conversion of plasminogen to plasmin can be inhibited by the following:
(1) Endogenous proteins
α2-macroglobulin
Plasminogen activator inhibitor type 1 protein
(2) Medications
Aminocaproic acid
Activation of plasminogen can be stimulated by the following:
(1) Endogenous proteins
Factors XI_a, XII_a
Kallikrien
Urokinase plasminogen activator
Tissue plasminogen activator
(2) Medications
Tissue plasminogen activators (e.g., alteplase, reteplase, tenecteplase)
Thrombolytics (e.g., urokinase, streptokinase)
Plasmin is directly inhibited by α2-antiplasmin and α2-macroglobulin
PLASMIN
FIBRIN CLOT
FIBRIN SPLIT PRODUCTS

FIGURE 17-4. Endogenous and exogenous factors that inhibit or activate plasminogen's conversion to plasmin.

TESTS TO EVALUATE HEMOSTASIS

For the purpose of discussion, bleeding and clotting disorders are organized by tests that assess platelets, coagulation, and clot degradation. Tests to assess platelets include platelet count, volume (e.g., mean platelet volume [MPV]), function (e.g., bleeding time [BT] and platelet aggregation), and others. Prothrombin time (PT)/international normalized ratio (INR), activated partial thromboplastin time (aPTT), activated clotting time (ACT), fibrinogen assay, thrombin time (TT), and others are laboratory tests that assess coagulation; a hypercoagulable panel can be drawn to determine whether or not a patient has one or more hypercoagulable disorders. Clot degradation is assessed with tests for FDPs and D-dimer.

In addition, general hematological values such as hemoglobin, hematocrit (Hct), red blood cell (RBC) count, and white blood cell (WBC) count, as well as urinalysis and stool guaiac tests may be important to obtain when evaluating blood and coagulation disorders; some of these tests are further discussed in Chapter 15. **Table 17-3** is a summary of common tests used to evaluate bleeding disorders and monitor anticoagulant therapy.

TABLE 17-3. Summary of Coagulation Tests for Hemorrhagic Disorders and Anticoagulant Drug Monitoring

DISORDER OR DRUG	PLATELET COUNT	PT/INR	APTT	COMMENTS
Thrombocytopenic purpura	Low	WNL	WNL	
Glanzmann thrombasthenia	WNL	WNL	WNL	Platelets appear normal
von Willebrand disease	Low or WNL	WNL	WNL or prolonged	Factor VIII levels low or WNL, vWF (antigen level and activity) low or WNL
Fibrinogen deficiency	WNL	Prolonged	Prolonged	BT prolonged if severe, fibrinogen levels decreased, TT prolonged
Warfarin therapy	WNL	Prolonged	WNL or prolonged	BT prolonged if overdosed
Unfractionated heparin therapy	WNL	WNL or prolonged	Prolonged	Platelet count may decrease
Vascular purpura	WNL	WNL	WNL	Normal platelet count distinguishes this from other forms of purpura such as TTP or ITP

aPTT= activated partial thromboplastin time; BT= bleeding time; ITP= idiopathic thrombocytopenic purpura; PT/INR= prothrombin time/international normalized ratio; TTP= thrombotic thrombocytopenic purpura; vWF= von Willebrand factor; WNL= within normal limits.

Platelet Tests

Platelet Count

Normal range: 150,000–450,000/μL (150–450 $\times 10^9$/L)

The only test to determine the number or concentration of platelets in a blood sample is the *platelet count,* through either manual (rarely done) or automated methods. Interferences with platelet counts include RBC fragments, platelet clumping, and platelet satellitism (platelet adherence to WBCs). Automated platelet counts are performed on anticoagulated whole blood. Most instrumentation that performs hematological profiles provides platelet counts. Platelets and RBCs are passed through an aperture generating an electric pulse with a magnitude related to the size of the cell/particle. The pulses are counted, and the platelets are separated from the RBCs by size providing the platelet count and MPV as well as the RBC count and mean corpuscular volume.

Thrombocythemia. An abnormal platelet count can have many causes. Thrombocythemia, also known as *thrombocytosis* or *elevated platelet count,* may be caused by the following factors:

- Stress
- Infection
- Splenectomy
- Trauma
- Asphyxiation
- Rheumatoid arthritis
- Iron deficiency anemia
- Posthemorrhagic anemia
- Cirrhosis
- Chronic pancreatitis
- Tuberculosis
- Occult malignancy
- Recovery from bone marrow suppression

Values of 500,000–800,000/μL are not uncommon. Thrombocythemia may be seen with any of the chronic myeloproliferative neoplasms, essential thrombocythemia, polycythemia vera, chronic myelogenous leukemia, or idiopathic myelofibrosis. Clinical consequences of thrombocythemia include thrombosis, hemorrhage, and microcirculatory disturbances. Thrombotic events may be either arterial or venous and include cerebrovascular accidents, myocardial infarction, deep venous thrombosis, pulmonary embolism, and intra-abdominal (portal and hepatic) vein thrombosis. Hemorrhagic complications usually involve the skin and mucous membranes, which include ecchymosis, epistaxis, and menorrhagia. Microcirculatory disturbances, such as headache, paresthesias, and erythromelalgia, may be due to microthrombi, which results in occlusion and ischemia. Additionally, patients with thrombocythemia may have abnormalities in platelet function studies, which can manifest as bleeding problems.

Thrombocytopenia. There are four main causes of thrombocytopenia, and patients may have more than one of these causes: (1) increased destruction or consumption of platelets; (2) decreased production; (3) dilution; and (4) sequestration.[6] Mucosal and cutaneous bleeding is the most common clinical consequence of thrombocytopenia; however, patients with only modest decreases in platelet counts may be asymptomatic. (**Minicase 1**.) When the platelet count falls below 20,000/μL, the patient is at risk of spontaneous bleeding. Therefore, platelet transfusions are often initiated. Bleeding may occur at higher platelet counts (e.g., 50,000/μL) if trauma occurs. The most common cause of death in a patient with severe thrombocytopenia is central nervous system (CNS) bleeding such as intracranial hemorrhage.

Numerous drugs have been associated with thrombocytopenia (**Table 17-4**).[7] However, heparin and antineoplastics are the most common ones implicated. Thrombocytopenia is also common with radiation therapy. Many drugs associated with thrombocytopenia alter platelet antigens resulting in the formation of antibodies to platelets (e.g., heparin, penicillin, and

MINICASE 1

Bleeding Disorders

Helen M., a 56-year-old female, is hospitalized with complaints of chest pain. She undergoes percutaneous coronary intervention (PCI) and is started on aspirin, clopidogrel, metoprolol, atorvastatin, and lisinopril.

The following laboratory parameters are obtained after PCI:

LABORATORY STUDY	NORMAL RESULTS	PATIENT'S TEST RESULTS
PT	10–13 sec	515.8 sec
INR	0.9–1.1	1.66
aPTT	21–45 sec	8080 sec
Platelet count	140,000–440,000/μL	67,000/μL
MPV	7–11 fL	14 fL

QUESTION: What specific test(s) have been performed to assess the pertinent patient findings from the history and physical examination? How might these tests relate to normal hemostasis? Does this patient need to undergo genotype testing prior to clopidogrel?

DISCUSSION: Her platelet count is decreased, and her MPV is increased, which may be due to the cardiac causes of her chest pain. Preliminary screening has been done using the PT/INR and aPTT; increased PT, INR, aPTT values are consistent with heparin given during the procedure. Studies indicate that genotype testing is not universally recommended. If she is started on a PPI for GI protection during hospitalization, she should be prescribed one with minimal CYP2C19 activity as a precaution for interaction with clopidogrel. Both aspirin and clopidogrel will affect platelet aggregation, and atorvastatin has been associated with thrombocytopenia.

TABLE 17-4. Partial List of Agents Associated with Thrombocytopenia

- Anti-infectives:
  - Acyclovir
  - Amphotericin B
  - Ampicillin
  - Ciprofloxacin
  - Clarithromycin
  - Ethambutol
  - Fluconazole
  - Isoniazid
  - Itraconazole
  - Linezolid
  - Oxacillin
  - Piperacillin
  - Quinine
  - Rifampin
  - Trimethoprim
  - Vancomycin
- Anti-seizure:
  - Carbamazepine
  - Phenobarbital
  - Phenytoin
  - Valproic acid
- Psychiatric:
  - Diazepam
  - Haloperidol
  - Lithium
- Cardiac:
  - Abciximab
  - Amiodarone
  - Atorvastatin
  - Clopidogrel
  - Digoxin
  - Eptifibatide
  - Hydrochlorothiazide
  - Low molecular weight heparin
  - Procainamide
  - Quinidine
  - Simvastatin
  - Tirofiban
  - Unfractionated heparin
- Pain:
  - Acetaminophen
  - Diclofenac
  - Ibuprofen
  - Naproxen
- Other:
  - Antineoplastics
  - Ethambutol
  - Interferon-α
  - Ranitidine

Source: See reference 7.

gold). Several diseases, such as thrombotic thrombocytopenic purpura (TTP), idiopathic thrombocytopenic purpura (ITP), disseminated intravascular coagulation (DIC), and hemolytic-uremic syndrome, result in rapid destruction of platelets. Other causes of thrombocytopenia include viral infections; pernicious, aplastic, and folate or B_{12}-deficiency anemias; complications of pregnancy; massive blood transfusions; exposure to DDT (dichlorodiphenyltrichloroethane); and human immunodeficiency virus (HIV) infections.

Heparin-induced thrombocytopenia (HIT) is an antibody-mediated adverse reaction to heparin, occurring in 1 in 5000 hospitalized patients, which may cause venous and arterial thrombosis.[8] Specifically, this is due to the development of IgG antibodies that bind to the heparin PF4 complex. Patients receiving UFH are generally at a higher risk of developing HIT than patients receiving LMWH, and it does not bind to PF4 as well as UFH, which is thought to be due to the smaller size of LMWH compared to UFH. Therefore, the heparin-PF4 complex is less likely to form with LMWH, and there are less IgG antibodies generated. The frequency or risk of HIT is influenced by certain factors such as heparin preparation, route, dose, and duration of heparin therapy, patient population, gender, and previous history of heparin exposure.[8] The animal source of heparin may also play a role in determining who develops HIT; bovine UFH seems to carry a higher risk compared to porcine UFH.[9]

The 4Ts score is a clinical prediction tool to determine the probability of HIT. This tool requires that the clinical evaluate the degree of thrombocytopenia, the timing of platelet count fall, the presence of thrombosis or other clinical sequelae, and other causes for thrombocytopenia; a score of 0–2 is assigned for each of the four items based on specific patient characteristics to determine the probability of HIT occurring in that particular patient. Thus, the score range is 0–8. A score of 0–3, 4–5, or 6–8 suggests a low, moderate, or high probability of HIT, respectively.[10] HIT is manifested both by clinical and serological features, and diagnosis of HIT is usually made when antibody formation is detected by an in vitro assay plus one or more of the following: unexplained decrease in platelet count (usually ≥30%, even if the nadir remains above 150 × 10^9/L), venous or arterial thrombosis, limb gangrene, necrotizing skin lesions at the heparin injection site, or acute anaphylactoid reactions occurring after intravenous (IV) heparin bolus administration.[10]

There are two types of tests to help diagnose HIT: (1) the enzyme-linked immunosorbent assay (ELISA), which identifies anti-PF4/heparin antibodies, and (2) functional assays, such as the C-serotonin release assay or the heparin-induced platelet activation assay—both of which detect antibodies that induce heparin-dependent platelet activation.[9-11] The ELISA test has high sensitivity and wide availability, with a relatively rapid turnaround time compared to the functional assays, which makes it a good screening test. However, the ELISA test has limited specificity so there may be false-positive results, especially in patients with antiphospholipid syndrome (APS) or systemic lupus erythematosus.[9,11] In contrast, the functional assays have high specificity, which are useful for confirming a positive ELISA test but are technically difficult and require the use of radioactivity and donor platelets.[9,11]

The typical onset for HIT is 5–10 days following the start of heparin; however, onsets occurring either earlier or later than this have been reported. Rapid-onset HIT occurs when platelet counts fall within 24 hours of heparin initiation, which is typically due to repeated heparin exposure within the past 100 days, and thus patients still have circulating HIT antibodies. Delayed-onset HIT, where thrombocytopenia occurs several days after discontinuation of heparin, has also been reported and is associated with DIC.[11] Platelet counts should be checked in patients receiving UFH or LMWH if the clinician deems the risk of HIT >1%; in these cases, recommendations are for platelet counts to be done every two to three days from days 4–14, or when heparin is stopped.[10] If the risk of HIT is <1%, then platelet monitoring is not recommended.[10] For patients who received heparin within the past 100 days, platelets should be checked at baseline and then within 24 hours of starting heparin.[10] **Table 17-5** outlines the patient characteristics associated with the risk of developing HIT.

If HIT is suspected and confirmed, UFH and LMWH should be discontinued. DTIs, such as argatroban or bivalirudin, can be used instead of UFH or LMWH. Both argatroban and bivalirudin are FDA-approved for use in patients with or at risk for HIT undergoing percutaneous coronary intervention (PCI); argatroban has an additional indication for the prophylaxis and treatment of thrombosis in patients with HIT.[12,13] Fondaparinux is an injectable, synthetic, indirect inhibitor of factor Xa. Although it is not FDA-approved for use in patients with HIT, there have been reports of successfully using fondaparinux as an alternative anticoagulant in the HIT population; however, there are also reports of fondaparinux-associated HIT or

TABLE 17-5. Incidence of HIT According to Patient Characteristics and Recommendations for Monitoring Platelets

RISK OF DEVELOPING HIT	>1%	<1%
Patient characteristics/examples	Postoperative patients on prophylactic dose or therapeutic dose UFH ≥4 days Cardiac surgery patients	Medical patients on prophylactic or therapeutic-dose UFH or LMWH ≥4 days Postoperative patients on prophylactic or therapeutic dose LMWH ≥4 days Patients receiving UFH flushes Obstetrics patients Intensive care patients
Frequency of platelet counts	Every 2–3 days from days 4–14, or until heparin is discontinued, whichever occurs first	Routine monitoring is not recommended

HIT = heparin-induced thrombocytopenia; LMWH = low molecular weight heparin; UFH = unfractionated heparin.
Source: See reference 10.

complications from using fondaparinux in patients with HIT.[14-20] The 9th edition of the *American College of Chest Physicians Evidence-Based Clinical Practice Guidelines* recommends the use of fondaparinux in HIT as a second-line agent to other parenteral DTIs in hospitalized patients or as a first-line parenteral agent in patients who develop an acute thrombosis unrelated to HIT as a bridge until warfarin therapy can be used.[10] In patients who require warfarin, it is recommended to wait to start warfarin until platelets have recovered to at least 150 × 10^9/L and to start at low doses.[10] The successful use of dabigatran, rivaroxaban, and apixaban in HIT has been limited to case reports or in vitro studies; thus, currently available data is insufficient to recommend the use of DOACs in managing HIT.[21] A prospective cohort study is underway in Canada looking at rivaroxaban in HIT, which may further elucidate the role of DOACs for this indication in the future.[22]

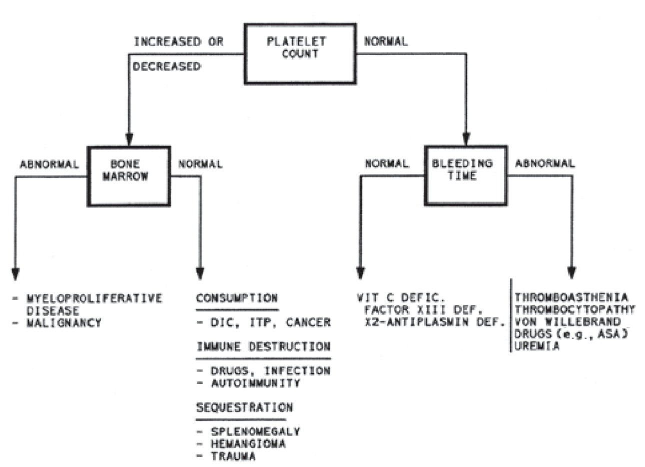

FIGURE 17-5. Assessment of abnormalities of homeostasis based on platelet count, bone marrow exam, and BT.

Mean Platelet Volume

Normal range: 7–11 fL (varies with laboratory)

Mean platelet volume (MPV)—the relationship between platelet size and count—is most likely to be used by clinicians in assessing disturbances of platelet production. MPV is useful in distinguishing between hypoproductive and hyperdestructive causes of thrombocytopenia (**Figure 17-5**). Despite the widespread availability of this platelet index, many clinicians do not use it in clinical decision making. In the past, this disuse was attributed to difficulties with the laboratory measurement of indices.

Many laboratories routinely report the MPV as part of the complete blood count, especially if a differential is requested. In general, lower platelet counts are common with higher platelet volumes, as an inverse relationship exists between the platelet count and the MPV. This inverse relationship correlates with platelet production within the bone marrow. Although MPV is most valuable in distinguishing hypoproductive from hyperdestructive causes of thrombocytopenia, a definitive diagnosis cannot be made based on MPV alone. In thrombocytopenia, an elevated MPV suggests no problem with platelet production, when in fact, production is reflexively increased. Conversely, a normal or low MPV suggests impaired thrombopoiesis. Determination of MPV requires a blood collection tube containing an anticoagulant. Usually, such tubes contain the anticoagulant ethylenediamine tetraacetic acid (EDTA), which causes an inflation of the MPV.

Currently, MPV is not widely used but may evolve into a valuable screening test for the disorders listed in **Table 17-6**.[23] For example, a fall in MPV is common in patients with enlarged spleens (hypersplenism) due to preferential sequestering of larger platelets within the spleen. An increase in MPV is seen during the third trimester of pregnancy in preeclamptic patients, where an increase in platelet size results from increased platelet consumption.[24] The MPV also is elevated in hyperthyroid patients but declines to normal as they become euthyroid.[25,26] A recent study also showed that patients with subclinical hypothyroidism also have an elevated MPV.[27]

The inverse relationship of a high MPV and a low platelet count is demonstrated in other conditions including respiratory disease, renal failure, and sepsis.[28-30] Unlike most other conditions that demonstrate the inverse relationship of MPV and platelet count, both are low in HIV infection. These decreases suggest an impairment of synthesis and maturation of megakaryocytes as well as enhanced platelet destruction in the bloodstream.[31]

TABLE 17-6. Conditions Associated with Alterations in MPV

Increase in MPV	Decrease in MPV
DM	Ulcerative colitis
HTN	HIV infection
Hyperlipidemia	Hypersplenism
PAD	Aplastic anemia
Myocardial infarction	Rheumatoid arthritis
Unstable angina	Systemic lupus erythematosus
Decompensated heart failure	
Ischemic stroke	
Venous thromboembolism	
Pre-eclampsia	
Rheumatoid arthritis	
Ankylosing spondylitis	
Psoriasis	
Pulmonary arterial hypertension	
Subclinical hypothyroidism	
Hyperthyroidism	
Celiac disease	
Renal failure	
Sepsis	
Erythropoietin	

DM = diabetes mellitus; HIV = human immunodeficiency virus; HTN = hypertension; MPV = mean platelet volume; PAD = peripheral arterial disease.

TABLE 17-7. Medications and Drug Classes That May Cause Abnormalities of Platelet Function

	ABNORMALITY	
MEDICATION	PROLONGED BT	ABNORMAL PLATELET AGGREGATION
Aspirin	√	√
β-blocking agents		√
Calcium channel blockers	√	√
Cephalosporins	√	√
Chemotherapeutic agents	√	√
Dextran	√	√
DOACs	√	
Ethanol	√	√
Heparin	√	√
Nitrofurantoin	√	√
Nitroglycerin	√	√
NSAIDs	√	√
Phenothiazines		√
$P2Y_{12}$ inhibitors	√	√
Quinidine	√	
Thrombolytic agents	√	
Warfarin	√	

DOACs = direct oral anticoagulants; NSAIDs = nonsteroidal anti-inflammatory drugs.
Source: See reference 36 for more information.

Administration of erythropoietin stimulates megakaryocyte cell line production that leads to an increase in MPV. Thrombopoietin probably causes the same effect.[32-35] The role of thrombopoietin has led to advances in the treatment of thrombocytopenia caused by deficient production of platelets (e.g., in patients undergoing bone marrow transplantation or cancer chemotherapy).[35]

Platelet Function

Abnormalities of *platelet function* may be either inherited or acquired. Bleeding as a result of an inherited versus acquired abnormality may be difficult to prove. Common bleeding sites in patients with disorders of platelet function include ecchymosis of the skin, epistaxis, gingival bleeding, and menorrhagia. Gastrointestinal hemorrhage and hematuria are less common and usually have an associated underlying pathology.[1] Hematomas and hemarthroses occur in patients with moderate-to-severe, inherited, familial-clotting disorders.[1]

Although the sites of bleeding may be predictable, the severity is not predictable in patients with inherited disorders of platelet function. Unfortunately, the risk of bleeding and bleeding patterns in patients with acquired platelet dysfunction are less predictable and more difficult to distinguish. Because both inherited and acquired etiologies increase the risk of bleeding, patients overtly bleeding without a clear cause or without an invasive procedure should be evaluated for one of these platelet function disorders. Simply stated, the platelet function tests look at the ability of platelets to aggregate and form a clot; platelet counts are usually normal. This can be due to medications, the platelet milieu, and inherent platelet defects.[6]

Bleeding time (normal range: two to nine minutes). BT is a measure of platelet function and has been used to assess bleeding risk, but this test is neither specific nor sensitive; thus, it does not help differentiate among the types of problems seen in disorders of primary hemostasis, such as von Willebrand disease and platelet function defects. This would account for its declining use and elimination by some institutional clinical laboratories. Additionally, the test is invasive and must be performed by a trained healthcare worker. To perform the test, small cuts are made on the forearm of the patient, and the time it takes to stop bleeding is measured. Several factors can prolong the BT including thrombocytopenia, certain medications, and conditions such as uremia and macroglobulinemia. Most acquired disorders affecting BT are related to medications that decrease platelet numbers or reduce platelet function. These include aspirin, $P2Y_{12}$ inhibitors (clopidogrel, prasugrel, ticagrelor), GPIIb/IIIa inhibitors (abciximab, eptifibatide, tirofiban), and phosphodiesterase inhibitors (dipyridamole). Other drugs that may prolong BT are listed in **Table 17-7.**[36] Although BT is influenced by some drugs, it is not used to monitor drug therapy. The increase in BT caused by aspirin may have beneficial effects in the treatment and prevention of cardiovascular disease. The proven value of aspirin for acute treatment of myocardial infarction and secondary prevention of cardiovascular disease is well established; however, the use of aspirin for primary prevention of cardiovascular disease remains an individual clinical judgment.

Platelet aggregation. With the many drawbacks of the BT, there was a need for a test that could aid in the diagnosis of defects in platelet function. This is especially true when considering the interpatient variability seen when taking clopidogrel. Adverse events may occur if patients do not respond to this medication. The ability of platelets to aggregate is most commonly measured by preparing a specimen of platelet-rich plasma and warming it to 98.6 °F (37 °C) with constant stirring. This test is performed with an aggregometer that measures light transmission through a sample of platelets in suspension. After a baseline reading is obtained, a platelet-aggregating agonist (e.g., epinephrine, collagen, ADP, or arachidonic acid) is added. As platelets aggregate, more light passes through the sample. The change in optical density can be measured photometrically and recorded as an aggregation curve, which is then printed on a plotter. Although light transmittance aggregometry (LTA) testing is the gold standard in platelet function analysis, it has requirements for specially trained personnel, large sample volume, and sample preparation; additionally LTA is expensive and has poor reproducibility.[37]

Interpretation of platelet aggregation tests involves a comparison of the patient's curves with the corresponding curves of a normal control. To eliminate the optical problems of turbidity with lipemic plasma, the patient and the normal control should be fasting. Patients should not take medications that affect platelet aggregation (e.g., aspirin, NSAIDs, P2Y$_{12}$ inhibitors) for approximately 7–14 days prior to the test because they may interfere with test results.

Novel point-of-care technologies are available, which allow for rapid and meaningful evaluation of platelet function, although major differences between different devices do exist.[37] These devices can assess the effects of medications such as aspirin, P2Y$_{12}$ inhibitors, and GP IIb/IIIa antagonists on platelet function and help predict the incidence of major adverse cardiac events in patients treated with medications affecting platelet function. [37] For example, the VerifyNow P2Y$_{12}$ device measures effects of P2Y12 inhibitors as "P2Y12 reaction units" or PRUs; a low PRU indicates a high percentage of inhibition, meaning the patient has a good response to this medication.[38] Further studies of each individual device are needed to elucidate the exact place in therapy of these in monitoring antiplatelet medications.

Other Platelet Tests

The measurement of platelet-specific substances, such as PF4 (normal values 1.7–20.9 ng/mL) and β-thromboglobulin (normal levels 6.6–47.9 ng/mL), can now be performed by radioimmunoassay or enzyme immunoassay.[39] High concentrations of these substances may be observed with coronary artery disease, acute myocardial infarction, and thrombosis, where platelet lifespan is reduced. Because numerous drugs can potentially cause thrombocytopenia, detection of antibodies directed by specific drugs against platelets may help to determine the culprit. Platelet survival can be measured by injecting radioisotopes that label the platelets. Serial samples can then determine platelet survival, which is normally 8–12 days.

Pharmacogenomics and clopidogrel metabolism. Genetic variability in the genes coding for CYP2C19 may have an effect on clopidogrel efficacy and safety. Carriers of the CYP2C19*2 genotype have a loss of function allele, which means these patients have reduced antiplatelet effect and are at increased risk for cardiovascular events, particularly stent thrombosis, when taking clopidogrel compared to patients without this genotype.[40] The product labeling for clopidogrel includes a warning about decreased effectiveness in poor metabolizers of the medication due to CYP2C19.[41] Carriers of the CYP2C19*17 genotype have a gain of function allele, which means these patients are at increased risk of bleeding compared to patients without this genotype. [40] There are commercially available assays to test for these variants in CYP2C19 although further studies are needed to show a clinical benefit.[42] The most recent AHA/ACC NSTE-ACS guidelines do not recommend routine genotype testing in all patients taking clopidogrel; instead, they recognize a possible role for testing patients who will have high-risk PCI procedures such as bifurcating left main artery.[43,44]

COAGULATION TESTS

Coagulation tests are useful in the identification of deficiencies of coagulation factors responsible for bleeding as well as thrombotic disorders. The most commonly performed tests, including the PT, INR, aPTT, and ACT, are used to monitor anticoagulant therapy. Numerous, high-precision automated laboratory methods are available to perform these tests. However, an overall lack of standardization across coagulation testing can lead to considerable variation in test results and their interpretation. Normal and therapeutic ranges established for one test method are not necessarily interchangeable with other methods, especially when differences in endpoint detection or reagents exist. Therefore, it is important to interpret test results based on the specific performance characteristics of the method used to analyze samples.

Coagulation studies may be used to assess certain bleeding disorders such as hemophilia A (factor VIII deficiency) or hemophilia B (factor IX deficiency). These deficiencies, which are inherited sex-linked recessive traits, primarily affect males and cause over 90% of hemophilia cases. Other bleeding disorders include von Willebrand disease—the most common hereditary bleeding disorder—and deficiencies in fibrinogen or factors II, V, VII, X, XI, XIII, and a combination of these factors.

Patients with thrombotic disorders may have their hypercoagulability evaluated with specific assays for the following[45]:

- APS—lupus anticoagulant, anticardiolipin antibody
- AT
- Protein C
- Protein S
- Prothrombin G20210A mutation
- Activated protein C (APC) resistance mutation (factor V Leiden)
- Homocysteine polymorphisms

These tests are often performed in panels because the presence of more than one predisposition to thrombosis further increases the risk for thrombosis. Normal reference ranges for AT and proteins C and S are often reported as a percent of normal activity, with 100% being the mean normal value. For AT, the normal activity level is 80–130%; for both proteins C and S, normal activity levels are 70–140%. Deficiencies can result in frequent, recurrent thromboembolic events in patients with these disorders. Because these deficiencies are rare, their respective assays are not discussed here in detail. Acquired, transient deficiencies of any of these inhibitors may be observed during thrombotic states. Therefore, these parameters should not be assessed during the acute phase of thrombosis or while the patient is currently on anticoagulant therapy because a false-positive result may occur. It is recommended to test for AT, protein C, protein S, and APC resistance after the thrombosis has been resolved when the patient is off of heparin or warfarin for a few weeks; the test for prothrombin G20210A mutation is not affected by current anticoagulant therapy.[45]

APC resistance, due to the factor V Leiden mutation, is the most prevalent hereditary predisposition to venous thrombosis. It is present in 3–5% of the general Caucasian population and is less common or rare in other ethnic groups. [45] It accounts for 20% of unselected patients with a first deep vein thrombosis and 50% of familial cases of thrombosis.[45] Patients with the heterozygous form of factor V Leiden mutation are at a fourfold to sevenfold higher risk of developing venous thromboembolism (VTE), while those with the homozygous form can be as high as 80-fold higher risk.[45] Prothrombin G20210A mutation is the second most common hereditary predisposition to venous thrombosis. DNA-based methods, such as the polymerase chain reaction-based assay, are used to determine the presence or absence of a specific mutation at nucleoside position 20210 in the prothrombin gene. A normal test would show absence of the G20210A mutation. The test identifies individuals who have the G20210A mutation and reveals whether the affected individual is heterozygous or homozygous for the mutation; patients with the heterozygous form are at a threefold higher risk of venous thrombosis, while those with the homozygous form are at an even higher risk.[45]

Anticoagulation therapy has traditionally consisted of an oral vitamin K antagonist (warfarin) with or without a parenteral agent such as UFH, LMWH, or fondaparinux. Within the last several years, several DOACs have come to the market, which have greatly increased the therapeutic options available for clinicians and patients. Advantages of DOACs compared to warfarin include fixed-dosing, fewer drug and dietary interactions, and little-to-no routine laboratory monitoring; disadvantages of DOACs compared to warfarin include increased cost, lack of reversal agent/antidote except for dabigatran, and less clinical experience with these newer agents. Although routine laboratory monitoring is not indicated with DOACs, there are some clinical instances when laboratory assessment could be considered, including thrombotic or hemorrhagic event, perioperative management, suspicion of overdosage/toxicity, renal/hepatic dysfunction, extremes of body weight, trauma, questionable adherence to therapy, concomitant administration with significant drug interactions, elderly patients, and following attempted reversal of anticoagulation.[46-49] Although therapeutic levels associated with optimal outcomes have not been established for the DOACs, "on-therapy" ranges have been proposed, which usually encompass the 5th through the 95th percentile concentration for a given DOAC; most patients at steady state will fall somewhere in this range during treatment with a DOAC.[42] Certain coagulation tests are better suited to assess qualitative (presence or absence of drug) versus quantitative (estimates of drug levels) information for specific DOACs, which are discussed in detail below.

Careful attention to blood collection technique, sample processing, and laboratory quality control is critical for reliable coagulation test results. Blood is collected in syringes or vacuum tubes that contain heparin, EDTA, or sodium citrate. Because heparin and EDTA interfere with several clotting factors, only sodium citrate is used for coagulation and platelet tests. Errors in coagulation can be significant unless quality assurance is strict concerning specimen collection, reagents, controls, and equipment. Factors that promote clotting and interfere with coagulation studies include the following:

- Tissue trauma (searching for a vein)
- Prolonged use of tourniquet
- Small-bore needles
- Vacuum tubes
- Heparin contamination from indwelling catheters
- Slow blood filling into collection tube

Bleeding risk and test results. The major determinants of bleeding are the intensity of the anticoagulant effect, the underlying patient characteristics, the use of drugs that interfere with hemostasis (**Tables 17-4, 17-7, and 17-8**), and the length of anticoagulant therapy. When evaluating anticoagulation treatment, one must weigh the potential for decreased thrombosis risk versus increased bleeding risk. The risk of bleeding associated with continuous IV heparin in patients with acute thromboembolic disease is approximately 5%. Some evidence suggests that this bleeding increases with an increase in heparin concentration. However, evidence also suggests that serious bleeding can occur in patients prone to bleeding even when the anticoagulant response is in the therapeutic range. The risk of bleeding is usually higher earlier in therapy, when both heparin and warfarin are given together, which may be related to excessive anticoagulation. Also, patients who have a coexisting disease that elevates the PT, aPTT, or both (e.g., liver disease) are often at much higher risk of bleeding. In these patients, the use and intensity of anticoagulation that should be employed are controversial.

Prothrombin Time/International Normalized Ratio

Normal range for PT: 10–13 sec but varies based on reagent-instrument combinations normal range for INR: 0.8–1.1; therapeutic range for INR depends on indication for anticoagulation; most indications: 2–3

The *prothrombin time* (PT), also called *ProTime,* test is used to assess the integrity of the extrinsic and common pathways (factors II, V, VII, X). The PT, based on the Quick method first described in 1935, is determined by adding calcium and a thromboplastin reagent containing both TF and phospholipid to the patient's plasma, and the time to clot formation is measured.[50] Deficiencies or inhibitors of extrinsic and common pathway clotting factors results in a prolonged PT; however, it should be noted that the PT is more sensitive to deficiencies in the extrinsic pathway (factor VII) compared to the common pathway (factors V, X, II, and fibrinogen).[51]

Assay performance characteristics, standardization, and reporting. The PT is dependent on the thromboplastin source and test method used to detect clotting. Thromboplastin reagents are derived from animal or human sources and include recombinant products. Factor sensitivity is highly dependent on the source of the thromboplastin, and can exhibit variability between different lots of the same reagent. Some thromboplastin reagents are less sensitive to changes in factor activity. This means that it takes a more significant decrease in factor activity to produce a prolongation of the PT. Differences in reagent sensitivity, combined with the influence of endpoint detection, affect clotting time results both in the normal and therapeutic ranges. Large differences in factor sensitivity between comparative methods can result in conflicting interpretation of results, both in the assessment of factor deficiencies and adequacy of anticoagulation therapy. Heparin also may prolong PT because it affects factor II in the common pathway; the addition of a heparin neutralizing agent to the blood sample can blunt this effect at heparin concentrations up to 2 units/mL.[51] However, at higher concentrations of heparin—whether due to higher doses of heparin or sample collection issues—the neutralizing agent may not be enough, and the PT may be prolonged. These "crossover" effects may have to be considered when oral and parenteral anticoagulants are given concomitantly for several days to avoid premature discontinuation of the parenteral agent. The PT is not as sensitive as the aPTT for dabigatran; PT levels may be normal or prolonged at on-therapy and above on-therapy dabigatran levels, so this is not a useful test for monitoring or measuring dabigatran levels.[49] In terms of the oral factor Xa inhibitors, the PT is more sensitive to edoxaban and rivaroxaban compared with apixaban; a normal or prolonged PT level suggests that rivaroxaban or edoxaban are at on-therapy or above on-therapy levels. [49] However for apixaban, prolonged levels usually indicate on-therapy or above on-therapy levels, but a normal PT does not exclude on-therapy levels for apixaban.[49]

Because PT results can vary widely depending on the thromboplastin source, the international normalized ratio (INR) is the standardized reporting method for monitoring warfarin therapy, which is known The INR is calculated according to the following equation:

$$\text{INR} = (\text{patient PT/mean normal PT})^{\text{ISI}}$$

The international sensitivity index (ISI) expresses the sensitivity of the thromboplastin reagent compared to the World Health Organization reference standard. The more sensitive or responsive the reagent, the lower the ISI. Theoretically, an INR result from one laboratory should be comparable to an INR result from a different laboratory, even though the PTs may be different. The citrate concentration also may affect the ISI determination of certain reagents, with higher citrate concentrations leading to higher INR results; using blood samples anticoagulated with 3.2% citrate, instead of higher concentrations, can help mitigate this problem. [50]

Although the INR system has greatly improved the standardization of the PT, one can still expect differences in INRs reported with two different methods, particularly in the upper therapeutic and supratherapeutic ranges. The greater the differences in the ISI values for two comparative methods, the more likely differences will be noted in the INR. Laboratories and anticoagulation clinics should review the performance characteristics of the PT method used to evaluate their specific patient populations and report changes in methods to healthcare professionals, particularly those monitoring anticoagulant therapy.

Monitoring warfarin therapy. Both the PT and INR may be reported when monitoring warfarin therapy, although clinically, only the INR is used to adjust therapy. Warfarin exerts its anticoagulant effects by interfering with the synthesis of vitamin K-dependent clotting factors (II, VII, IX, and X) and the natural anticoagulant proteins C, S, and Z. Specifically, warfarin inhibits vitamin K-reductase and vitamin K epoxide-reductase (VKOR), which blocks the activation of vitamin K to its reduced form. Reduced vitamin K is needed for the carboxylation of clotting precursors of factors II, VII, IX, and X. Noncarboxylated clotting factor precursors are nonfunctional, and thus an anticoagulated state is achieved.[50] Warfarin is manufactured as a racemic mixture of (S)- and (R)-enantiomers; the S-enantiomer is more potent than the R-enantiomer at inhibiting VKOR, which is why the S-enantiomer is responsible for the majority of the anticoagulant effects of warfarin. The S-enantiomer is metabolized largely by CYP2C9, while the R-enantiomer is metabolized mostly by CYP1A2, and CYP3A4; other CYP enzymes also are involved in the metabolism of warfarin although to a lesser extent.

Current *American College of Chest Physicians Evidence-Based Clinical Practice Guidelines* recommend an INR of 2–3 for most indications. A higher INR of 2.5–3.5 is recommended for, but is not limited to, patients with mechanical prosthetic heart valves in the mitral position and patients with recurrent thromboembolic events.[52-56] Results below the therapeutic range indicate that the patient is at increased risk for clotting, and warfarin doses may need to be increased. Results above the therapeutic range indicate the patient is at risk for bleeding and warfarin doses may need to be decreased. Numerous drugs, disease states, and other factors prolong the INR in patients receiving warfarin by various mechanisms of action (**Table 17-8**).

Pharmacogenomics and oral anticoagulant therapy. Genetic variability in the genes coding for CYP2C9, VKORC1 (vitamin K epoxide reductase complex subunit 1), CYP4F2, and NQO1 can influence warfarin dosing by altering its pharmacokinetics and pharmacodynamics.[57,58] CYP2C9 and VKORC1 have a larger influence compared to CYP4F2. Specifically, patients with CYP2C9*2 and CYP2C9*3 variations have a reduced clearance of the (S)-warfarin enantiomer, which results in lower maintenance dose requirements of warfarin, increased risk of bleeding,

TABLE 17-8. Factors Altering Pharmacokinetics and Pharmacodynamics of Warfarin

ANTICOAGULANT EFFECT POTENTIATED	ANTICOAGULANT EFFECT COUNTERACTED
Low vitamin K intake	Increased vitamin K intake
Reduced vitamin K absorption in fat malabsorption	
Drug interactions:	Drug interactions:
Acetaminophen	Azathioprine
Amiodarone	Barbiturates
Anabolic steroids	Carbamazepine
Cimetidine	Cholestyramine
Clarithromycin	Rifampin
Disulfiram	Alcohol (chronic consumption)
Erythromycin	Nafcillin
Fenofibrate	
Fluconazole	
Isoniazid	
Metronidazole	
fluoroquinolones	
Phenytoin	
Piroxicam	
Quinidine	
Tamoxifen	
Trimethoprim–sulfamethoxazole	
Heart failure exacerbation	
Liver disease	
Pyrexia	
Thyrotoxicosis	
Alcohol (acute consumption or binge drinking)	

Source: See reference 50.

and a possible longer time to achieve a stable dosing regimen.[57] Two main haplotypes of VKORC1, low-dose haplotype group A, seen predominantly in Asian patients, and high-dose haplotype group B, seen predominantly in African-American patients, contribute to the interindividual variability of warfarin dosing. The specific single nucleotide polymorphisms (SNPs) involved are the -1639G>A and 1173C>T. Patients with the AA genotype (predominately Asians) require lower doses of warfarin compared to Caucasians, while patients with the GG genotype (predominantly African Americans) require higher doses compared to Caucasians.[57] The CYP4F2 enzyme normally plays a role in the conversion of vitamin K to vitamin KH_2, which is needed to carboxylate the clotting factor precursors; patients with a polymorphism in the 433Met allele of the CYP4F2 gene will have greater vitamin K availability leading to higher warfarin dose requirements.[57] The Hispanic population has been largely underrepresented in warfarin pharmacogenomic studies. Smaller studies have shown that CYP4F2 and NQO1 genotypes significantly contributed to warfarin dose requirements in a population of inner-city, Hispanic Americans; the genotypic contributions of CYP2C9 and VKORC1 in Hispanic Americans were similar to non-Hispanic Caucasians.[58,59] Unlike CYP2C9 polymorphisms, neither VKORC1 nor CYP4F2 have been associated with increased bleeding risks or prolonged time to achieve a stable dose of warfarin.[57]

Five manufacturers are currently marketing their warfarin pharmacogenomics testing devices; each one tests for the CYP2C9*2 and CYP2C9*3, as well as either the VKORC1 −1639G>A or the VKORC1 1173C>T SNPs.[42] The product labeling for Coumadin (warfarin) has been updated to include information about the potential impact of pharmacogenomics on the dosing of this medication as well as a pharmacogenetics dosing table, which may help clinicians select an initial dose of warfarin if genetic information is known, specifically in regard to CYP2C9 and VKORC1.[60] For clinicians who do utilize genetic testing for their patients on warfarin, they can use either the dosing table provided in the package insert or a dosing algorithm to estimate a starting dose for their patient. Algorithms take into account not only the results of genetic testing, but also other factors such as age, body size, smoking status, use of other medications like amiodarone, other disease states, and vitamin K intake. Subsequent dosing changes should be made based on results of the INR test.

There are several barriers to widespread adoption of pharmacogenetic testing and dosing: unavailability of testing at many medical centers, which leads to outsourcing of tests and a long turnaround time for results; lack of reimbursement for testing leading to large out-of-pocket expenses for patients; conflicting results from clinical trials; conflicting guidelines regarding genetic testing by professional organizations; and a lack of clinician acceptance and knowledge of interpreting and applying test results. Clinical studies have provided mixed results when comparing genotype-guided dosing to conventional dosing. The COAG study showed no differences in percentage of time in therapeutic range (TTR) at four weeks when genotype information was available to assist in dosing versus clinically guided dosing algorithms.[61] However, the EU-PACT study showed a significantly higher percentage of TTR with genotype-guided dosing compared to usual care.[62] A meta-analysis showed that genotype-guided dosing did not show improvements in % TTR, supratherapeutic INRs, rates of major or minor bleeding, rates of thromboembolism, or all-cause mortality in the first month of warfarin therapy. After one month, there were improvements in %TTR and incidence of major bleeding with genotype-guided dosing.[63] The ongoing GIFT study is looking at possible differences in clinical outcomes (thrombotic events, major bleeding events) instead of a surrogate marker such as %TTR.[64]

The 9th edition of the *American College of Chest Physicians Evidence-Based Clinical Practice Guidelines* recommends against the routine use of pharmacogenetic testing when initiating a patient on warfarin.[53] However, the *Clinical Pharmacogenetics Implementation Consortium Guidelines* do recommend using available genotypic information to aid in warfarin dosing.[65] Genetic testing, if utilized, should be used along with patient characteristics, clinical considerations, and continued INR monitoring for optimal outcomes associated with warfarin use.

Although genetic variants have not been well studied regarding the DOACs, there are some potential genes that may influence a patient's response to these medications. The CYP enzymes are not important to the metabolism of dabigatran, although the CYP 3A4/5 and CYP2J2 do play a role in the metabolism of rivaroxaban and may serve as a cause of genetic variability in patients using rivaroxaban; both dabigatran and rivaroxaban are P-glycoprotein substrates, which is encoded by the ABCB1 gene where several SNPs have been identified.[56] A subset of patients enrolled in the RE-LY trial underwent genotype testing with results showing that SNPs on the CESI and ABCB1 genes were associated with bleeding; further studies are needed to determine if these SNPs are associated with ischemic events.[66] Edoxaban was shown to have little interpatient variability due to genetic variations in the factor X gene.[67] Further studies are needed to elucidate potential genetic influences in the dosing of DOACs.

Activated Partial Thromboplastin Time

Normal range: varies by manufacturer, generally between 25–35 sec; therapeutic range for heparin-treated patients is 1.5–2.5 times control aPTT

The *activated partial thromboplastin time* (aPTT) is used to screen for deficiencies and inhibitors of the intrinsic pathway (factors VIII, IX, XI, and XII) as well as factors in the final common pathway (factors II, V, and X). The aPTT also is commonly used as a surrogate assay to monitor UFH and DTIs. The aPTT, reported as a clotting time in seconds, is determined by adding an aPTT reagent, containing phospholipids and activators, and calcium to the patient's blood sample.

Factor and heparin sensitivity as well as the precision of the aPTT test depend both on the reagents and instrumentation. In addition, some aPTT reagents are formulated for increased sensitivity to lupus anticoagulants. Despite numerous attempts to standardize the aPTT, very little progress has been made. The difficulty in part may reflect differences in opinion as to the appropriate heparin sensitivity, the need to have lupus anticoagulant sensitivity for targeted patient populations, and suitable factor sensitivity to identify deficiencies associated with increased bleeding risk. Normal and therapeutic ranges must be established for each reagent instrument combination, and ranges should be verified with changes in lots of the same reagent. Laboratory errors may cause either prolongation or shortening of the aPTT; these may include an inappropriate amount and concentration of anticoagulant in the collection tube, time between collection of the blood specimen and performance of the assay, inappropriate collection site (i.e., through a venous catheter, which contains heparin), and inappropriate timing of blood collection.[51]

Causes of aPTT prolongation. In addition to reagent specific issues impacting on aPTT responsiveness, hereditary diseases, or other acquired causes may prolong aPTT test results. Causes of aPTT prolongation include the following:

- Hereditary Causes
    1. Deficiency of factor VIII, IX, XI, XII, prekallikrein, or high-molecular weight kininogen (HMWK) (PT is normal)
    2. Deficiency of fibrinogen or factor II, V, or X (PT also is prolonged)

It should be noted that aPTT reagents may respond differently if a patient has a single factor deficiency versus a multiple factor deficiency; patients with single factor deficiency have a more predictable aPTT prolongation compared to patients with multiple factor deficiencies.[68]

- Acquired Causes
    1. Lupus anticoagulant (PT usually normal)
    2. Heparin (PT less affected than aPTT; PT may be normal)
    3. Bivalirudin, or argatroban (PT usually also prolonged)
    4. Dabigatran (less accurate at higher dabigatran concentrations)
    5. Liver dysfunction (PT affected earlier and more than aPTT)
    6. Vitamin K deficiency (PT affected earlier and more than aPTT)
    7. Warfarin (PT affected earlier and more than aPTT)
    8. DIC (PT affected earlier and more than aPTT)
    9. Specific factor inhibitors (PT normal except in the rare case of an inhibitor against fibrinogen, factor II, V, or X)
    10. Decreased nutritional intake; malabsorption
    11. Myeloproliferative disease

Use of aPTT to monitor heparin. Although used to detect clotting factor deficiencies, the aPTT is used primarily for monitoring therapeutic heparin therapy and is emerging as a potential way to qualitatively monitor dabigatran therapy. The generally accepted therapeutic range of heparin is an aPTT ratio of 1.5–2.5 times control, although this has not been confirmed by randomized trials.[69] Given the interpatient and intrapatient variability that can result from aPTT reagents, alternative means of monitoring heparin therapy are being scrutinized. This 1.5–2.5 aPTT ratio corresponds to the following[69]:

- A plasma heparin concentration of 0.2–0.4 units/mL by assay using the protamine titration method
- A plasma heparin concentration of 0.3–0.7 units/mL by assay using the inhibition of factor Xa

UFH should be given by continuous IV infusion or subcutaneous injection, with exact dosing dependent on the indication. The aPTT should be drawn at baseline, four to six hours after continuous IV heparin is begun, and four to six hours after each subsequent dosage adjustment, because this interval approximates the time to achieve steady-state levels of heparin. Institutions may have their own specific heparin dosing nomogram or base their nomogram off one used in clinical studies; using a nomogram also allows quick fine-tuning of anticoagulation by nurses without continuous physician input.

aPTT determinations obtained earlier than six hours, when a steady-state concentration of heparin has not been achieved, may be combined with heparin concentrations for dosage individualization using non-steady-state concentrations. This approach has been demonstrated to reduce the incidence of subtherapeutic aPTT ratios significantly during the first

24 hours of therapy.[70,71] This finding is important because the recurrence rate of thromboembolic disease increased when aPTT values were not maintained above 1.5 times the patient's baseline aPTT during the first 24 hours of treatment.[72,73]

Heparin concentration measurements may provide a target plasma therapeutic range, especially in unusual coagulation situations such as pregnancy, where the reliability of clotting studies is questionable. In this setting, shorter than expected aPTT results in relation to heparin concentration measurements may be indicative of increased circulating levels of factor VIII and increased fibrinogen levels.[74] Patients may have therapeutic heparin concentrations measured by whole blood protamine sulfate titration or by the plasma anti-Xa heparin assay. However, they may have aPTTs not significantly prolonged above baseline. This difference has been referred to as a dissociation between the aPTT and the heparin concentration.[75] Many of these patients have very short pretreatment aPTT values.

Current recommendations for patients with decreased aPTT results on heparin are that such patients be managed by monitoring heparin concentrations using a heparin assay to avoid unnecessary dosage escalation without compromising efficacy. These patients, referred to as *pseudoheparin resistant*, may be identified as having a poor aPTT response (to an adequate heparin concentration >0.3 units/mL via plasma anti-Xa assay) despite high doses of heparin (>50,000 units/24 hr; usual dose is 20,000–30,000 units/24 hr). When higher doses of heparin (>1500 units/hr) are required to maintain therapeutic aPTT values, high concentrations of heparin-binding protein or phase reactant proteins bind and neutralize heparin. Additionally, thrombocytosis, or AT deficiency may exist.

Another use for heparin concentrations is to demonstrate both efficacy and safety with LMWH, which have several indications. However, clinically, the anti-factor Xa levels are more routinely used for this class of medications. LMWH has a pharmacokinetic and pharmacodynamic profile, which makes routine monitoring unnecessary in most circumstances. Exceptions include special populations, such as those patients with renal failure or severe obesity who are at risk of being overdosed when weight-adjusted regimens are used. Both PT and aPTT times are not significantly prolonged at recommended doses of LMWHs.[89,109] However, both efficacy and safety can be demonstrated by assaying anti-factor Xa levels. This assay is recommended to be drawn four hours after administration of a therapeutic weight-adjusted dose of LMWH, when anti-factor Xa activity has peaked. An effective plasma concentration range is approximately 0.5–1.1 plasma anti-Xa units/mL for twice-daily subcutaneous dosing of LMWH. The effective plasma concentration for once daily dosing of LMWH is less certain but has been recommended to be approximately 1–2 plasma anti-Xa units/mL.

Decreased aPTT levels. Although most attention has been focused on causes of prolonged aPTT levels, there is growing evidence of adverse events associated with decreased aPTT levels, including VTE, MI, hyperthyroidism, diabetes, spontaneous abortion, and death.[76] Clotting factors of the intrinsic pathway as well as vWF levels and activity have been elevated in some patients presenting with decreased aPTT levels, which provides some evidence that patients with decreased aPTT levels are hypercoagulable.[77] There is no definitive answer whether a shortened aPTT is a cause, consequence, or just an association with these other conditions. To rule out whether a shortened aPTT is due to a laboratory error, such as inappropriate specimen collection, a repeat collection and repeat testing should be performed.

Heparin alone has minimal anticoagulant effects; when it is combined with AT (normal range: 80–120%), the inhibitory action of AT on coagulation enzymes is magnified 1000-fold resulting in the inhibition of thrombus propagation. Patients who are AT deficient (<50%) may be difficult to anticoagulate, as seen with DIC (**Minicase 2**). The DIC syndrome is

MINICASE 2

A Case of DIC

Teresa G., a 36-year-old female in her third trimester of pregnancy, is hospitalized with clinical suspicion of DIC because of acute onset of respiratory failure, circulatory collapse, and shock. The following laboratory values for her are obtained:

LABORATORY RESULTS	NORMAL RESULTS	PATIENT RESULTS
PT	10–13 sec	16 sec
aPTT	25–35 sec	59 sec
TT	25–35 sec	36 sec
Hgb	12.3–15.3 g/dL	9.8 g/dL
Hct	36–45%	27.7%
Platelet count	150,000–450,000/μL	64,000/μL
MPV	7–11 fL	17 fL
FDP (latex)	<10 mcg/mL	120 mcg/mL
AT	80–120%	57%
D-dimer	<200 ng/mL	2040 ng/mL

QUESTION: What laboratory tests are used to determine if a patient is experiencing DIC? What are the expected laboratory results for these tests?

DISCUSSION: Laboratory findings of DIC may be highly variable, complex, and difficult to interpret. Both PT and aPTT should be prolonged (and they are prolonged in this patient), but this may not always occur. Because of this, the usefulness of both PT and aPTT determinations may be helpful in making the diagnosis. TT is prolonged as expected. The platelet count is typically and dramatically decreased. Her MPV is inversely related to her decreased platelet count as expected, suggesting a hyperdestructive phenomenon versus a hypoproliferative state. Although FDPs are elevated, this rise is not solely pathognomonic for DIC. Increased D-dimer levels are strongly suggestive of DIC. AT determination reveals a considerable decrease consistent with DIC. Decreased AT is useful and reliable for diagnosis of DIC in the absence of D-dimer testing ability.

associated not only with obvious hemorrhage but also with occult diffuse thrombosis.

Oral anticoagulant effect on aPTT. Although warfarin mildly elevates the aPTT, aPTT is not used to monitor warfarin therapy. Therefore, if warfarin is started in a patient receiving heparin, the clinician should expect some elevation in aPTT. On-therapy or above on-therapy levels of dabigatran can prolong the aPTT, but a normal aPTT does not exclude on-therapy or below on-therapy levels; thus the aPTT provides qualitative information about dabigatran but not quantitative information.[49,68] The aPTT is even less sensitive than the PT for the oral factor Xa Inhibitors and thus cannot be recommended for either qualitative or quantitative assessment for these agents; on-therapy levels of these medications cannot be excluded with a normal aPTT.[49]

Activated Clotting Time

Normal range: 70–180 sec but varies

Activated clotting time (ACT), also known as *activated coagulation time,* is frequently used to monitor heparin or DTIs when very high doses are required, such as during invasive procedures like cardiopulmonary bypass graft surgery, percutaneous transluminal coronary angioplasty, PCI extracorporeal membrane oxygenation, valve replacements, or carotid endarterectomy. The ACT also can be used to monitor heparin neutralization following protamine administration during these types of surgeries, although a return to baseline ACT does not indicate full neutralization.[78] In most cases, an ACT is obtained from a point-of-care machine using whole blood; thus, it may be run directly in the operating room as well as at the bedside when a rapid heparinization is required (e.g., hemodialysis unit, operating room, and cardiac catheterization laboratories).

ACT responsiveness remains linear in proportion to an increasing dose of heparin, whereas the aPTT has a log-linear relationship to heparin concentration. Corresponding ACT values up to 400 seconds demonstrate this dose-response relationship, but ACT lacks reproducibility for values in excess of 600 seconds as well as low concentrations of heparin. ACT test results can be influenced by the following factors[79]:

- Testing device
- Testing technique
- Sample temperature
- Hemodilution
- Platelet count and function
- Factor deficiencies
- Hypothermia
- Lupus anticoagulants

There is a wide correlation when comparing results of the aPTT and ACT, which suggests that these tests are not equivalent and may result in dissimilar clinical decisions.[80] Given the lack of advantages over aPTT monitoring of heparin for treatment of VTE, the ACT is not recommended for use in this setting. The main indication to use the ACT over the aPTT is for a patient receiving high dose heparin or DTIs. The DOACs will prolong the ACT, but reproducibility is poor for the factor Xa inhibitors, and sensitivity is low for dabigatran; thus, this test is not recommended for qualitative or quantitative measurements of these agents.[68]

Anti-Xa

Normal range: varies based on specific anticoagulant used for treatment of existing VTE

- *Heparin: 0.3–0.7 IU/mL*
- *LMWH: 0.5–1 IU/mL (twice daily dosing); 1–2 IU/mL (once daily dosing)*
- *Fondaparinux, rivaroxaban, apixaban, edoxaban: not established*

The *anti-Xa* level may be used to monitor LMWH when given in therapeutic doses; however, routine monitoring is not usually done because LMWH has a more predictable dose-response relationship than UFH. Monitoring anti-Xa levels can be considered in patients with poor renal function, pregnant patients, neonates and infants, patients with cirrhosis, and patients with extremes in body weight.[69,81] Levels should be drawn four hours after the LMWH injection, otherwise subtherapeutic or supratherapeutic levels may occur. The anti-Xa level may be used to measure on-therapy and above on-therapy levels for rivaroxaban, apixaban, and edoxaban, but dabigatran has no effect on anti-Xa levels.[49,82] When ordering an anti-Xa test, it is imperative that the correct calibrator is used to ensure correct results; for example, the LMWH calibrator cannot be used to measure anti-Xa activity of fondaparinux. Most laboratories at this time do not have specific calibrators for the oral factor Xa inhibitors, which limit the usefulness of this test.[49,68] (**Minicase 3**.)

Fibrinogen Assay

Normal range: 200–400 mg/dL (5.8–11.8 μmol/L)

Although the PT and aPTT are used to screen for deficiencies in the intrinsic, extrinsic, and common pathways, the *fibrinogen assay* is most commonly used to assess fibrinogen concentration. Fibrinogen assays are performed by adding a known amount of thrombin to a dilution of patient plasma. The fibrinogen concentration is determined by extrapolating the patient's clotting time to a standard curve. Elevated fibrinogen levels may be related to pregnancy or acute phase reactions, and may be associated with an increased risk of cardiovascular disease.[83] Decreased fibrinogen is associated with DIC and hepatic cirrhosis; PT and aPTT levels also may be increased due to decreased fibrinogen levels, and patients may have symptomatic bleeding. Additionally, supratherapeutic heparin concentrations >1 unit/mL may result in falsely low fibrinogen concentration measurements. TT(discussed below) is the most sensitive test for fibrinogen deficiency, and it is prolonged when fibrinogen concentrations <100 mg/dL. However, the actual fibrinogen concentration occasionally must be determined. Fibrinogen levels are usually drawn as part of a DIC panel, when further exploring the reasons for an elevated PT or aPTT level, or to further evaluate unexplained bleeding in a patient.

Thrombin Time

Normal range: 17–25 sec but varies according to thrombin concentration and reaction conditions

The *thrombin time* (TT), also known as *thrombin clotting time,* measures the time required for a plasma sample to clot after the addition of bovine or human thrombin and is compared to that of a normal plasma control. Deficiencies in both the intrinsic and extrinsic systems do not affect TT, which assesses only the

MINICASE 3

A Patient on Anticoagulants

Emily F., a 46-year-old female with type 2 diabetes mellitus and hypertension, presents to the emergency department with signs and symptoms of a new PE. One dose of weight-based LMWH is given. She weighs 45 kg and is 63 inches tall with normal renal function. She has the following laboratory determinations performed four hours after initiation of LMWH:

LABORATORY RESULTS	NORMAL RESULTS	PRETREATMENT PATIENT RESULTS	POST-LMWH RESULTS
PT (sec)	10–13	10.5	12.9
INR	0.9–1.1	1	1.2
anti-Xa (units/mL)	0.3–0.7	0.5	0.9
Protein C (%)	70–140	62	59

QUESTION: What might account for this patient's decreased protein C activity? Why was the anti-Xa level drawn? What anticoagulation strategies are potential options for her?

DISCUSSION: She was started on a LMWH with plans to convert to warfarin, per the institution's protocol. Protein C activity is decreased due to the acute thrombotic state so this should not infer that she has one or more of the hypercoagulable disorders. This type of test should not be done in the time period surrounding an acute event or during anticoagulant therapy as both instances can result in a false positive result.

Anti-Xa levels are not routinely drawn in most patients, but she falls into the lower extreme of body weight, so it may be prudent to see if her dose is correct. No significant effects were seen on any of her other laboratory results, which is consistent with LMWH.

Once warfarin therapy is initiated, either this same day or the next day, INR should be followed, with the desired endpoint of warfarin therapy being an INR of 2–3. She can be reassessed after a minimum of three months of warfarin therapy to see if further treatment is warranted. She can stop therapy and have a hypercoagulable panel assessed a few weeks later along with a D-dimer test to help decide her future anticoagulation needs.

QUESTION: Six months later, she presents for a routine follow up of her INR. D-dimer testing reveals that she is at risk for a recurrent event, and the decision is made to stay on warfarin therapy. She has not had many issues while on warfarin, and her INR has remained fairly stable. However, today she states she has a new job that requires a lot of travel, so frequent visits to the anticoagulation clinic will be difficult. What are her options for future anticoagulation?

DISCUSSION: One of the drawbacks to warfarin therapy is the need for frequent INR monitoring to ensure safety and efficacy of the medication. She may be able to transition to patient self monitoring or patient self-testing depending on if an INR meter will be covered by her insurance, whether she is capable of performing and assessing the test on her own and whether her anticoagulation clinic is able to fully support this type of management. Another option would be to transition to one of the DOACs if this is covered by her insurance and she does not have any contraindications to this type of medication. This would mean she would not have to watch her dietary consumption of vitamin K as much, nor would she have to come in for routine monitoring. She should be counseled, however, that there is not an antidote currently available for most of the DOACs as there is with warfarin.

final phase of the common pathway or essentially the ability to convert fibrinogen to fibrin.

Prolongation of TT may be caused by hypofibrinogenemia, dysfibrinogenemia, heparin, DTIs, or the presence of FDPs.[6] The TT is ultrasensitive to heparin and dabigatran; therefore, it only is useful to show whether or not these drugs are present in the blood sample—not as a monitoring test or to quantify drug levels. With thrombolytic therapy, laboratory monitoring may not prevent bleeding or ensure thrombolysis. However, some clinicians recommend measuring TT, fibrinogen, plasminogen activation, or FDPs to document that a lytic state has been achieved. Typically, TT is >120 seconds four to six hours after "adequate" thrombolytic therapy.

The dilute thrombin time (dTT) is a test that compensates for the extreme sensitivity of the TT to heparin and dabigatran by diluting the patient's blood sample with normal plasma. There is a commercially available dTT (HEMOCLOT), but overall the dTT is not widely available in most laboratory tests. Neither the TT nor dTT is a useful monitoring test for rivaroxaban, apixaban, or edoxaban.

Ecarin Clotting Time

The *ecarin clotting time* (ECT) test is a specific assay for thrombin generation. It is used to monitor parenteral DTIs and has been postulated as a way to monitor oral DTIs like dabigatran (**Table 17-9**). Ecarin is added to plasma which cleaves prothrombin to meizothrombin, a serine protease similar to thrombin. DTIs inhibit meizothrombin so the ECT can quantify the amount of DTI in the body by measuring the time for meizothrombin to convert fibrinogen into fibrin. Thus, a longer ECT corresponds to larger drug concentrations. ECT is not affected by other anticoagulants such as warfarin, heparin, or factor Xa inhibitors. The ECT is not routinely used to monitor dabigatran as this test it is not widely available and the test has not been standardized or validated.[48,49]

Clot Degradation Tests

Clot degradation tests are useful in assessing the process of fibrinolysis. These tests include FDPs and D-dimer, which can be used in the diagnosis of DIC or thrombosis and in monitoring the safety and efficacy of thrombolytic therapy. Thrombolytics (e.g., alteplase, reteplase, and tenecteplase) are exogenous agents

TABLE 17-9. Summary of Interpreting Laboratory Tests with DOACs

	TESTS THAT MAY BE USED FOR QUALITATIVE ASSESSMENT OF DOACs	TESTS THAT MAY BE USED FOR QUANTITATIVE ASSESSMENT OF DOACs	TESTS NOT RECOMMENDED
DTIs (dabigatran)	aPTT: prolonged TT: prolonged	ECT: not widely available, not standardized	PT: normal to prolonged Anti-Xa: no effect
Factor Xa inhibitors (apixaban, edoxaban, rivaroxaban)	PT: normal to prolonged (more sensitive for edoxaban and rivaroxaban)	Anti-Xa: no currently available standardized ranges	aPTT: normal to prolonged TT: no effect ECT: no effect

aPTT = activated partial thromboplastin time; DTIs = direct thrombin inhibitors; ECT = ecarin clotting time; PT = prothrombin time; TT = thrombin time.

that lyse clots already formed. They are used in the treatment of acute cerebrovascular accidents (CVA), myocardial infarction, VTE, and peripheral arterial occlusion. The mechanism by which they activate fibrinolysis can variably impact circulating proteins (hence, the necessity for close monitoring to minimize bleeding complications and ensure efficacy). Numerous laboratory parameters have been evaluated for this purpose, including PT, aPTT, BT, fibrinogen, FDPs, and D-dimer. These laboratory parameters are discussed throughout this chapter and in **Minicase 4.**

Fibrin Degradation Products

Normal range: <10 mcg/mL or <10 mg/L but varies with assay

Excessive activation of thrombin leads to overactivation of the fibrinolytic system and increased production of *fibrin degradation products* (FDPs). Excessive degradation of fibrin and fibrinogen also increases FDPs. This increase can be observed with DIC or thrombolytic drugs. FDPs can be monitored during thrombolytic therapy, but they may not be predictive of clot lysis. False-positive reactions may occur in healthy women immediately before and during menstruation and in patients with advanced cirrhosis or metastatic cancer.

D-Dimer

Normal range: <0.5 mcg/mL (<3 nmol/L) but varies with specific assay

D-dimer is a marker of thrombotic activity and is formed when thrombin initiates the transition of fibrinogen to fibrin and activates factor XIII to cross link the fibrin formed; when plasmin digests the cross-linked fibrin, d-dimer is formed. The D-dimer test is specific for fibrin, whereas the formation of

MINICASE 4

A Patient on Thrombolytic Therapy

Alfred F., a 44-year-old male, has clinical signs and symptoms and electrocardiogram findings consistent with acute anterior-wall myocardial infarction requiring PCI. However, he presents to a hospital without PCI capabilities. He receives reteplase between the transit time to the nearest hospital with PCI capability over two hours. Subsequently, he is started on a heparin infusion. The following pretherapy and posttherapy coagulation laboratory results are obtained:

LABORATORY STUDY	NORMAL RESULTS	PRETHERAPY	POST-THERAPY
PT	10–13 sec	12.2 sec	17 sec
aPTT	25–35 sec	35 sec	69 sec
Fibrinogen	200–400 mg/dL	300 ng/mL	22 ng/mL
FDP (latex)	<10 mcg/mL	<10 mcg/mL	>160 mcg/mL
D-dimer	<0.5 mcg/mL	<0.5 mcg/mL	<0.6 mcg/mL
Plasminogen	80–120%	70%	22%

QUESTION: What might explain the elevated FDP? What accounts for the fall in the plasminogen level on completion of the lytic therapy? Finally, why is the D-dimer concentration not elevated in proportion to the greatly elevated FDP concentration?

DISCUSSION: The elevated post-therapy PT and aPTT are consistent with heparin therapy after receiving reteplase. The FDP concentration is elevated because reteplase resulted in fibrinogenolysis. Many FDPs are generated in this setting. By the nature of thrombolytic therapy, plasminogen is converted to plasmin, accounting for the decline in the plasminogen percentage. Because thrombolytic therapy was unsuccessful in full clot lysis (with predominate fibrinogenolysis), the D-dimer concentration is not greatly elevated. For this assay to have been more elevated, degradation products arising from cross-linked fibrin (fibrinolysis) would have had to be present. Fibrinogen concentrations should be followed periodically in patients receiving thrombolytic agents.

DIAGNOSTIC FOLLOW-UP: If TT, PT, or aPTT is prolonged and if circulating inhibitors or bleeding disorders are suspected, further tests are usually performed. These may include assays for specific clotting factors to determine if a specific deficiency exists. For example, hemophilia or autoimmune diseases may be associated with inhibitors such as anti-factor VIII and the lupus anticoagulant.

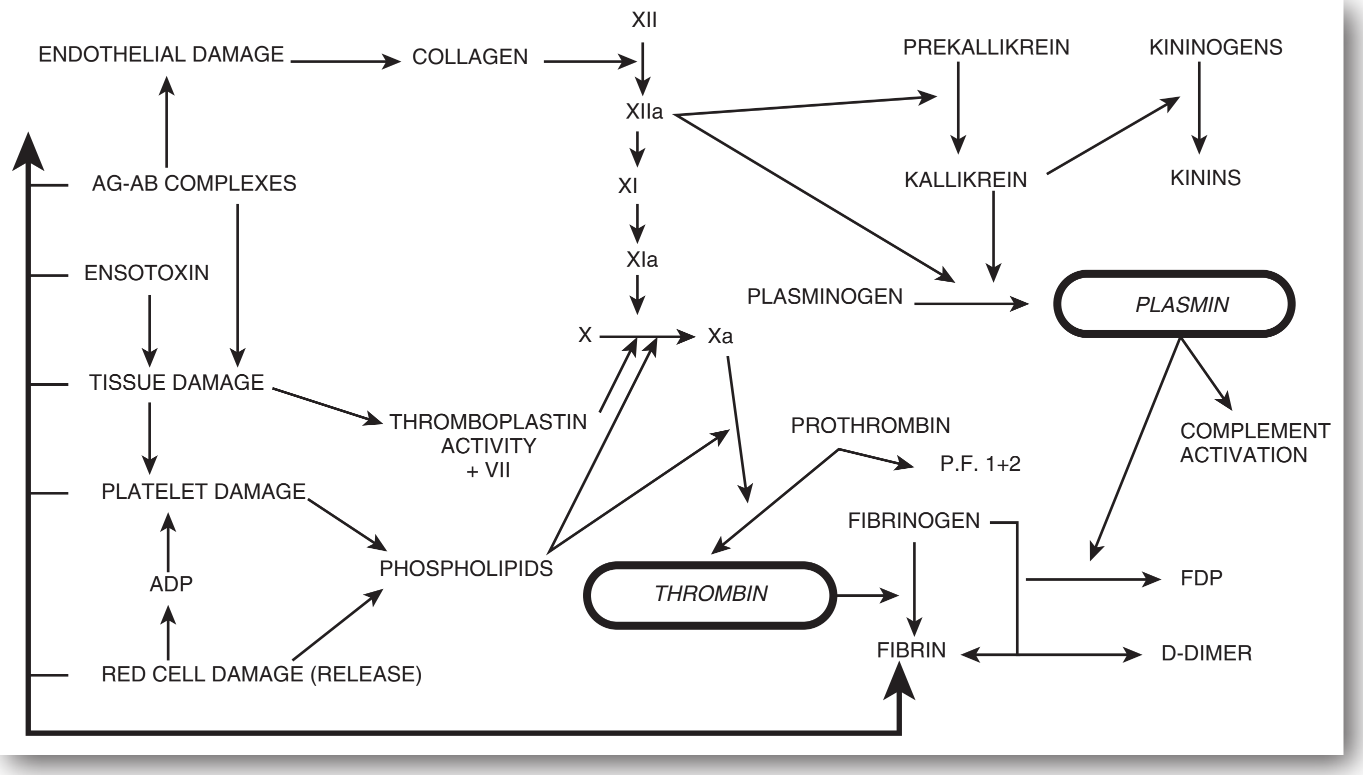

FIGURE 17-6. Formation of D-dimers in disseminated intravascular coagulation triggered by amniotic fluid embolism. *Source:* Reprinted from Bick RL. Disseminated intravascular coagulation: objective criteria for clinical and laboratory diagnosis and assessment of therapeutic response. *Semin Thromb Hemost.* 1996; 22:69-88. Copyright © 1996. SAGE Publications, Inc.

FDPs (discussed previously) may be either fibrinogen or fibrin derived following plasmin digestion (**Figure 17-6**).

The D-dimer is often used to help diagnose or rule out thrombosis in the initial assessment of a patient suspected of having acute thromboembolism; results are typically elevated if a patient is positive for VTE. However, D-dimer is a sensitive but nonspecific marker for VTE because other causes such as malignancy, DIC, infection, inflammation, and pregnancy also can elevate the D-dimer levels. Thus, a positive result does not necessarily confirm a diagnosis of VTE, but a negative result can help rule out VTE. Clinical correlation is essential, and further diagnostic workup is warranted with a positive test result to rule out other disorders as causes for abnormal levels. The 9th edition of the *American College of Chest Physicians Evidence-Based Clinical Practice Guidelines* recommends that a D-dimer test may be used as one possible diagnostic aid in patients with low or moderate probability for first-event VTE.[85]

In addition to diagnose or rule out VTE, D-dimer has been used for its predictive value for recurrent thromboembolism in patients treated for first event idiopathic VTE. Studies have shown that patients with normal levels of D-dimer after treatment for first event idiopathic VTE have a low risk for VTE recurrence, whereas elevated levels of D-dimer are predictive of VTE recurrence.[86-90] Thus, in patients with elevated levels of D-dimer, an extended duration of anticoagulation therapy could be considered.

D-dimer is also a common test used as an aid to diagnose and evaluate patients with DIC. Several scoring systems for DIC based on clinical and laboratory data have been developed, which include specific laboratory measurements such as platelet counts, PT, fibrinogen, and FDPs.[91] The clinical and laboratory results are given specific scores, and when added up, indicate whether a patient is likely to have DIC. Although a D-dimer test is not specifically mentioned in some scoring systems, it is often used as a fibrin degradation marker, and it is a simple and quick test to perform. **Table 17-10** provides a

TABLE 17-10. Laboratory Differential Diagnosis of DIC

MONITORING PARAMETER	DIC	PRIMARY FIBRINOLYSIS	TTP	CHRONIC LIVER DISEASE
FDP	↑	↑	WNL to ↑	WNL to ↑
D-dimer	↑	↑	WNL	WNL
PT	↑	↑	WNL	↑
aPTT	↑	↑	WNL	WNL to ↑
Fibrinogen	↓	↓	WNL	Variable
Platelet count	↓	WNL	WNL to ↓	↓
LFTs	WNL	WNL	WNL	↑
BUN	↑	WNL	↑	WNL

↓ = decreased; ↑ = increased; aPTT = activated partial thromboplastin time; BUN = blood urea nitrogen; DIC = disseminated intravascular coagulation; FDP = fibrin degradation product; LFTs = liver function tests; PT = prothrombin time; TTP = thrombotic thrombocytopenic purpura; WNL = within normal limits.

list of the laboratory parameters, including the D-dimer, used to diagnose DIC. (Minicases 3 and 4.)

Near-Patient or Point-of-Care Testing Devices

Several *point-of-care testing devices* are available for different coagulation tests, such as PT/INR, ACT, D-dimer, and platelet function tests. By design, point-of-care coagulation testing methods are easy to use by multiple healthcare professionals, adaptable to a number of patient care environments, require minimal to no sample processing, and are an extension of central laboratory testing when rapid turnaround is required. Like central laboratory testing, none of the PT, aPTT, and ACT methods are standardized and users are required to verify performance characteristics for normal and therapeutic ranges. In addition, users should have an understanding of the potential limitations due to sample type, sample stability, and volume. Although some instruments perform only one assay, a number of devices are capable of performing multiple tests. These devices typically are used in hospital settings and may be more cost effective than single assay platforms because hospitals can standardize all point-of-care coagulation testing using one system. Generally, point-of-care testing is not as precise as a fully automated central laboratory system that requires minimal user intervention, although in most cases the imprecision is an acceptable compromise for rapid turnaround.

Clinicians should have quality control measures in place to ensure the reliability of results; this includes ensuring that the testing device and components are properly working, personnel operating the device have adequate training, and results obtained via point-of-care testing are verified to be similar to those obtained via traditional venipuncture and analyzed in a laboratory.[78] Point-of-care testing uses whole blood, a sample that may be more physiologically relevant and result in a more accurate assessment of true coagulation potential. Many of these devices have some type of data management system that can interface with the health system's information system. The more advanced systems can restrict operator access, store specific information, manage quality control functions, and flag out-of-range results. These systems are usually reliable, but system performance and accuracy of results are reliant on routine maintenance and adherence to manufacturers' guidelines on test procedures and operational environment. A quality assurance program that utilizes recommended quality control materials and troubleshooting measures is essential to all clinical programs using point-of-care devices.

Point-of-care coagulation testing offers specific clinical advantages, especially when used to monitor antithrombotic therapy because test results can be combined with clinical presentation to make more timely decisions regarding therapeutic intervention. This is especially significant in emergency departments, cardiac catheterization laboratories, surgical settings, and critical care units where immediate turnaround time is essential to patient care decisions. With newer antithrombotic options, these technologies will become increasingly relevant and may aid in making decisions for major bleeding events and prior to emergent surgery. Although many of the newer drugs do not require routine monitoring, the availability of rapid interventional testing may be critical to the selection of certain therapies for target patient populations, particularly when these drugs have a long half-life or cannot be completely reversed. Concomitant therapy is being used increasingly in cardiac patients, especially during cardiac intervention; thus, the potential for thrombotic or hemorrhagic problems may be increased without the ability to rapidly confirm coagulation status, both at the initiation of therapy and at the conclusion of a procedure.

In outpatient settings, point-of-care testing may not only be clinically beneficial, but it is also more cost effective and convenient than central laboratory testing, particularly in oral anticoagulation clinics and home healthcare settings. Patients can be informed of their INR results and subsequent dosing instructions within minutes, which is an obvious time-saving element. Certain patient variables may limit the accuracy of results obtained from the currently available point-of-care devices for INR monitoring. These include concurrent use of LMWH or UFH, presence of antiphospholipid antibodies, and Hct levels above or below device-specific boundaries.

Patient self-testing (PST) and patient self-management (PSM) for INR are options for properly selected and trained patients on long-term warfarin therapy. PST is when a patient tests their own INR but relies on a clinician for interpretation of results and any modifications to the current regimen. PSM is when the patients test their own INR and adjust their own therapy, usually based off an algorithm, which offers more patient autonomy and control over their own dosages. Studies have shown that PST and PSM, when compared with standard care, had a reduced or comparable risk of thromboembolism or death and comparable rates of major bleeding.[92-95] Improvements in patient satisfaction and quality of life were seen in studies comparing PST/PSM to usual care as well.[93,96] Although there are benefits to a PST/PSM model of care, including increased convenience to the patient, there also are issues that limit the widespread use of PST and PSM in the United States. These include reimbursement from insurance companies, lack of large scale randomized trials utilizing a U.S. population, low levels of awareness or understanding among healthcare practitioners and patients about these options, and inability to operate the testing device.[97,98] Additionally, the cost-effectiveness of PST/PSM is not well defined. Higher costs are associated with the cost of the test strip as well as increased testing frequency, but this may be offset by the convenience of PST/PSM, especially for patients who live far away from testing facilities, those who have difficulty with scheduled appointments, or those who frequently travel.[53,97] Appropriate patient selection is essential for PST/PSM to be effective; ideal patient candidates or their caregivers should have manual dexterity, visual acuity, mental ability to perform the test, confidence and ability to responsibly participate in self-care, and the ability to complete a structured training course.[97]

SUMMARY

Many factors contribute to normal hemostasis, including interactions among vascular subendothelium, platelets, coagulation factors, natural anticoagulant proteins C and S, and substances that promote clot degradation such as tPA. In the clinical setting, the impact of these and other considerations must be evaluated. Disorders of platelets or clotting factors can result in bleeding, which may necessitate the monitoring of specific clotting tests.

Coagulation tests such as aPTT, ACT, and PT/INR are used to monitor heparin and warfarin therapies. The introduction of DOACs into clinical practice may see new standards for monitoring in the future as more data are collected about these agents. In general, coagulation tests are used for patients receiving anticoagulants, thrombolytics, and antiplatelet agents. The availability of rapid diagnostic tests to manage LMWH, DTIs, and platelet inhibitor drugs may influence the selection of these newer therapies. Other indications for routine use of these tests include primary coagulopathies and monitoring of drugs that may cause bleeding abnormalities. Finally, other available tests (e.g., D-dimer and AT level determinations) might improve diagnostic assessment of patients with DIC and ensure appropriate treatment selection.

LEARNING POINTS

1. ***What is the INR in relation to PT?***

   ANSWER: PT results are not standardized as various reagent sources and test methods are used when performing this test among differing laboratories. The INR is a calibration method developed to standardize the reported PT results. The method considers the sensitivity of individual reagents as well as the clot detection instrument used, designated as ISI. Although INR reporting has improved the standardization of the PT, there still remains potential problems (e.g., ISI calibration, sample citrate concentration, etc.). Thus, laboratories should review the performance of their individual methods used in reporting PT/INRs and inform clinicians who are interpreting these results of any changes.

2. ***How often should one monitor platelets after the initiation of UFH in the prevention of HIT?***

   ANSWER: HIT is an antibody-mediated adverse reaction to heparin, which can cause arterial and venous thrombosis. It is estimated to occur in 1–5% of patients receiving UFH. Platelet counts should be checked often in patients receiving UFH; the exact frequency depends on several factors. For patients receiving therapeutic doses of UFH for treatment of venous or arterial thrombosis, platelets should be monitored every two to three days until day 14 or until UFH is stopped. In those patients receiving UFH for postoperative prophylaxis, platelets should be monitored every two to three days between days 4 and 14 or until UFH is stopped. For patients who have received heparin within the past 100 days or in whom exposure history is uncertain, platelets should be checked at baseline and then within 24 hours of starting UFH.

3. ***Which laboratory tests may be used to monitor the DOACs?***

   ANSWER: There is not one single test that can universally monitor the DOAC medications. Certain tests are better suited for qualitative measurements, which show whether the medication is present in the body or not, while other tests are better suited for quantitative measurements, which show actual levels of medication in the body. Some of these tests are widely available, while others are not commonly available or results may not be available in a timely manner. Qualitative measurements of dabigatran can be done through the aPTT, while quantitative measurements of dabigatran can be done with either the dTT or the ECT. Qualitative information for the oral factor Xa inhibitors can be measured using the PT, while anti-Xa levels can be used for quantitative information.

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QUICKVIEW | Platelet Count

PARAMETER	DESCRIPTION	COMMENTS
Common reference ranges		
Adults	150,000–450,000/µL (150–450 × 10^9/L)	
Critical value	>800,000 or <20,000 µL (>800 × 10^9/L or <20 × 10^9/L)	
Inherent activity?	Determines the number or concentration of platelets in a blood sample	
Location		
Production	Bone marrow	Also can be produced by lungs and other tissues
Storage	Not stored	⅔ found in circulation, ⅓ found in spleen
Secretion/excretion	Destroyed by spleen, liver, bone marrow	
Causes of abnormal values		
High	Acute hemorrhage	
	Iron deficiency anemia	
	Diseases: splenectomy, rheumatoid arthritis, occult malignancy, myeloproliferative neoplasms	
Low	Hypersplenism	
	Severe B_{12}, folate deficiency	
	Diseases: TTP, ITP, DIC, aplastic anemia, myelodysplasia, leukemia	Table 17-3
	Drugs	
Signs and symptoms		
High	Thrombosis: CVA, DVT, PE, portal vein thrombosis	
Low	Bleeding: mucosal, cutaneous	CNS bleeding (i.e., intracranial hemorrhage) is the most common cause of death in patients with severe thrombocytopenia
After event, time to...		
Initial elevation	Days to weeks	
Peak values	Days to weeks	
Normalization	Weeks to months	
Causes of spurious results	Values outside 50,000–500,000/µL (50 × 10^9/L–500 × 10^9/L)	Need to review the peripheral blood smear to confirm automated platelet counts in these instances
	Hct <20 or >50%	

CNS = central nervous system; CVA = cerebrovascular accident; DIC = disseminated intravascular coagulation; DVT = deep vein thrombosis; Hct = hematocrit; ITP = idiopathic thrombocytopenic purpura; PE = pulmonary embolism; TTP = thrombotic thrombocytopenic purpura.

QUICKVIEW | PT and INR

PARAMETER	DESCRIPTION	COMMENTS
Common reference ranges		
Adults	PT 10–13 sec INR 0.8–1.1	INR therapeutic ranges will vary if patient is on warfarin and depending on indication for warfarin; usual therapeutic ranges are either 2–3 or 2.5–3.5
Pediatrics	PT <16 sec	PT levels in the newborn are generally prolonged compared to adults; however, by 6 mo of age, levels are comparable to adults
Critical value	PT >15 sec	Unless on warfarin
	INR—depends on indication, but >5 is commonly used as a critical value	
Inherent activity	Indirect measure of coagulation factors, particularly factor VII, which has the shortest half-life and thus is affected the most rapidly by warfarin	
Location		
Production	Coagulation factors produced in liver	
Storage	Not stored	
Secretion/excretion	None	
Causes of abnormal values		
High	Diseases: liver disease	Table 17-8
	Malabsorption/malnutrition	
	Drug: warfarin	
Low	None	Table 17-8
Signs and symptoms		
High	Increased risk of bleeding and ecchymosis	Risk increases as PT or INR value increases
Low	Potential thrombosis if on vitamin K antagonist	
After event, time to…		
Initial elevation	6–12 hr	
Peak values	Days to weeks	
Normalization	Hours–days	Depends, if reversed with vitamin K
Causes of spurious results	Improper laboratory collection	
Additional info	Used to monitor warfarin	Target levels depend on indication for warfarin

INR = international normalized ratio; PT = prothrombin time.

QUICKVIEW | aPTT

PARAMETER	DESCRIPTION	COMMENTS
Common reference ranges		
Adults	25–35 sec	May vary by reagent/instrument used
Critical value	>100 sec, but depends on specific reagent	
Inherent activity?	Used to monitor unfractionated heparin activity	If patient is on unfractionated heparin for treatment of deep venous thrombosis or pulmonary embolism, aim for 1.5–2.5 times control aPTT
Location		
Production	Coagulation factors produced in liver	
Storage	Not stored	⅔ found in circulation, ⅓ found in spleen
Secretion/excretion	None	
Causes of abnormal values		
High	Hereditary: deficiency of factors II, V, VIII, IX, X, XI, XII, HMWK, prekallikrein, fibrinogen	
	Acquired: lupus anticoagulant, heparin, DTIs, liver dysfunction, vitamin K deficiency, warfarin, DIC	aPTT not used to monitor warfarin therapy
Low	Laboratory testing drawn before 6 hr if on heparin, ATIII deficiency	
Signs and symptoms		
High	Increased risk of hemorrhage	Risk increases as aPTT increases
Low	Thrombosis	
After event, time to…		
Initial elevation	6–12 hr	
Peak values	Hours to days	
Normalization	Hours to days	
Causes of spurious results	Improper laboratory collection	Need to do manual counts in these instances
Additional info	Used to monitor heparin	Dosing nomograms vary by institution

aPTT = activated partial thromboplastin time; DIC = disseminated intravascular coagulation; DTIs = direct thrombin inhibitors; HMWK = high-molecular weight kininogen.

QUICKVIEW | anti-Xa

PARAMETER	DESCRIPTION	COMMENTS
Common reference ranges		
Adults	0.3–0.7 IU/mL, for unfractionated heparin 0.5–1 IU/mL for twice-daily LMWH 1–2 IU/mL for once-daily LMWH	For establishment of therapeutic heparin range using blood samples from heparinized patients Values may vary depending on LMWH preparation used
Inherent activity?	Used to establish therapeutic heparin range and monitor LMWH activity	
Location		
Production	Coagulation factors produced in liver	
Storage	Not stored	
Secretion/excretion	None	
Causes of abnormal values		
High	Overdosage of LMWH Poor renal function	
Low	Laboratory testing drawn prior to 4 hr after dose is administered Underdosage of LMWH	
Signs and symptoms		
High	Increased risk of bleeding and bruising	
Low	Potential thrombosis	
After event, time to...		
Initial elevation	0–4 hr	
Peak values	4 hr	
Normalization	Hours to days	
Causes of spurious results	Improper timing of collection	Should be drawn 4 hr after dose is administered
Additional info	Used to monitor LMWH	

LMWH = low molecular weight heparin.

18

INFECTIOUS DISEASES

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OBJECTIVES

After completing this chapter, the reader should be able to

- **List the common tests utilized by the microbiology laboratory for the identification of bacteria**
- **Describe the types of clinical specimens that may be submitted for Gram stain and culture**
- **Discuss the processes involved in staining and culturing a clinical specimen for bacteria, including the time required to obtain a result from either method; describe the clinical utility of the information obtained from a Gram stain or a culture**
- **Identify bacteria according to Gram stain results (gram-positive versus gram-negative), morphology (cocci versus bacilli), and growth characteristics (aerobic versus anaerobic)**
- **Define normal flora; identify anatomic sites of the human body where normal flora are commonly present and those that are usually sterile; identify bacteria that are considered normal flora in each of these sites**
- **Describe the most common causative pathogens based on infection type or anatomic site of infection**
- **Describe the common methods used for antimicrobial susceptibility testing including technique, type of result, clinical implications, and limitations of each method; demonstrate the**

(continued on page 422)

The assessment, diagnosis, and treatment of a patient with an infection may appear to be an overwhelming task to some clinicians. This may be partly due to the nonspecific presentation of many infectious processes; the continuously changing taxonomy, diagnostic procedures, and antimicrobial susceptibility patterns of infecting organisms; and the continuous, albeit diminishing, introduction of new antimicrobials to the existing large collection of anti-infective agents. This chapter focuses on the laboratory tests utilized for the diagnosis of infectious diseases. It is important to note that diagnostic tests for many infectious diseases, particularly the diagnosis of human immunodeficiency virus (HIV) infection, are continuously being modified to reflect technological advances in laboratory procedures.

This chapter describes some of the laboratory tests utilized in the diagnosis of the most common infections due to bacteria, fungi, mycobacteria, viruses, and other organisms. Information regarding white blood cells (WBCs) and their role in infection is discussed in Chapter 17. Laboratory tests utilized in the diagnosis of viral hepatitis, *Helicobacter pylori* gastrointestinal (GI) infection, and *Clostridium difficile* pseudomembranous colitis are addressed in Chapter 15. Lastly, information regarding the clinical utility of the erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) as they relate to inflammatory diseases are addressed in Chapter 19; however, this chapter will provide a brief discussion regarding their clinical utility in the diagnosis and management of infectious diseases.

BACTERIA

Bacteria are small, unicellular, prokaryotic organisms that contain a cell wall but lack a well-defined nucleus. They are a diverse group of microorganisms that exist in different shapes and morphologies with varying rates of pathogenicity. Bacteria are a common cause of infection in both the community and hospital setting and can cause infection in patients with normal or suppressed immune systems. Bacteria must be considered potential causative pathogens in any patient presenting with signs and symptoms of infection.

The Identification of Bacteria

Several factors should be considered when choosing an appropriate antimicrobial regimen for the treatment of infection, including patient characteristics (e.g., immune status, age, end-organ function, drug allergies, and severity of illness), drug characteristics (e.g., spectrum of activity, pharmacokinetics, penetration to the site of infection, and proven clinical efficacy), and infection characteristics such as the site/type of infection (suspected or known) and potential causative organism(s). Therefore, appropriate diagnosis is a key factor in selecting appropriate empiric and directed antibiotic therapy for the treatment of an infection. In the case of a suspected infection, appropriate culture specimens should be obtained for laboratory testing from the suspected site of infection *before* antibiotics are initiated, if possible, in an attempt to isolate and identify the causative pathogen. Special attention should be placed on specimen collection and timely transport to the laboratory because the accuracy of the results will be limited by the quality and integrity of

OBJECTIVES

ability to appropriately use susceptibility information when choosing an antimicrobial regimen for a patient

- Define minimum inhibitory concentration (MIC), MIC_{50}, MIC_{90}, MIC susceptibility breakpoints, and minimum bactericidal concentration
- Describe the information that is utilized to construct a cumulative antibiogram; discuss the clinical utility of the cumulative antibiogram when choosing empiric antibiotic therapy for the treatment of a patient's infection
- Understand the basic methods that may be utilized in the diagnosis of systemic fungal infections
- Discuss the laboratory tests that are commonly utilized in the diagnosis of infections due to *Mycobacterium tuberculosis* and nontuberculous mycobacteria
- Discuss the laboratory tests that are commonly utilized in the diagnosis of common viral infections such as influenza, herpes simplex virus, cytomegalovirus, and respiratory syncytial virus
- Discuss the laboratory tests that are commonly utilized in the diagnosis of human immunodeficiency virus; describe the laboratory tests that are commonly utilized in the assessment and monitoring of patients with human immunodeficiency virus infection
- Understand the laboratory tests that may be performed for the diagnosis of infections due to miscellaneous or uncommon organisms such as *Borrelia burgdorferi*, *Treponema pallidum*, *Legionella pneumophila*, and *Pneumocystis (carinii) jirovecii*
- Understand the clinical utility of laboratory tests routinely performed for the diagnosis of infection in the following:
  a. Cerebrospinal fluid when meningitis is suspected
  b. Respiratory secretions when upper or lower respiratory tract infections are suspected
  c. Urine, prostatic secretions, or genital secretions when a genitourinary tract infection is suspected
  d. Otherwise sterile fluid when infection is suspected (e.g., synovial fluid, peritoneal fluid)

TABLE 18-1. Common Biologic Specimens Submitted for Culture[1-4]

Abscess, lesion, wound, pustule—swab or aspirate

Blood

Bone marrow

Body fluids—amniotic, abdominal, bile, pericardial, peritoneal, pleural, or synovial by needle aspiration

Bone—biopsy of infected area

CSF—by lumbar puncture or directly from shunt

Cutaneous—hair and nail clippings, skin scrapings, aspiration of leading edge of skin infection; biopsy

Ear—middle ear fluid specimen by myringotomy; outer ear specimen by swab or biopsy

Eye—swab of conjunctiva, corneal scrapings, vitreal or anterior chamber fluid

Foreign bodies—intravenous catheter tip by roll plate method; prosthetic heart valve, prosthetic joint material, intrauterine device, etc.

Gastrointestinal—gastric aspirate for AFB, gastric biopsy for *H. pylori*, rectal swab for VRE, stool cultures, stool specimen for *Clostridium difficile* toxin

Genital tract—cervical, endometrial, urethral, vaginal, or prostatic secretions; ulcer biopsy

Respiratory tract—sputum, tracheal aspirate, BAL, pharyngeal or nasopharyngeal swab, sinus aspirate

Tissue—biopsy specimen

Urine—clean catch midstream, catheterized, suprapubic aspirate

AFB = acid-fast bacilli; BAL = bronchoalveolar lavage; CSF = cerebrospinal fluid; VRE = vancomycin-resistant enterococci.

the submitted specimen.[1-4] **Table 18-1** lists common biologic specimens that may be submitted to the microbiology laboratory for bacteriologic analysis.[1-4]

When a specimen from the suspected site of infection is submitted to the microbiology laboratory, a number of microbiologic tests are performed to aid in identification of the infecting bacteria. The most common laboratory tests used for the identification of bacteria include direct microscopic examination using specialized stains (e.g., the Gram stain, fluorescent stains such as acridine orange or auramine-rhodamine stains) and bacterial culture techniques to foster growth of the microorganism. When bacteria grow in culture, additional tests are then performed to identify the infecting organism and to determine the susceptibility of the bacteria to various antimicrobial agents.

The Gram Stain

The *Gram stain* is the most common staining method utilized for the microscopic examination of bacteria and is most appropriate for evaluation of fluids (cerebrospinal fluid [CSF], pleural, synovial, etc.), respiratory tract secretions, and wound/abscess swabs or aspirates.[4] The Gram stain classifies bacteria into one of two groups, gram-positive or gram-negative, based on their reaction to an established series of dyes and decolorizers. The difference in stain uptake between gram-positive and gram-negative bacteria is primarily due to differences in their bacterial cell wall composition and permeability.[5-8] Although the Gram stain does not provide an exact identification of the infecting organism (e.g., *Klebsiella pneumoniae* versus *Serratia marcescens*), it does provide rapid (within minutes) preliminary information about the potential infecting organism that can be used to guide empiric antibiotic therapy while waiting for culture results, which may take 24–48 hours or more. The Gram stain is useful for characterizing most clinically relevant bacteria, but is unable to detect intracellular bacteria (e.g., *Chlamydia*) bacteria without cell walls (e.g., *Mycoplasma*) and organisms that are too small to be visualized with light microscopy (e.g., spirochetes).[6-8]

The current Gram stain methodology is a slight modification of the original process developed by Hans Christian Gram in the late 19th century.[5-8] The Gram stain procedure involves a number of staining and rinsing steps that can all be performed within a few minutes. The first step involves applying, drying, and heat-fixing a thin smear of a biological specimen to a clean glass slide. Once the slide has cooled, it is then rinsed with crystal or gentian violet (a purple dye) followed by Gram's iodine, decolorized with an ethanol or acetone rinse, and then counterstained with safranin (a pink or red dye) with a gentle, tap water rinse performed between each of these steps. The slide is then blotted dry and examined under a microscope using the oil immersion lens. If bacteria are present, they are examined for stain uptake, morphology (round = coccus, rod = bacillus), and organization (e.g., pairs, clusters). Gram-positive bacteria stain purple due to retention of the crystal violet-iodine complex in their cell walls, while gram-negative bacteria stain red because they do not retain crystal violet and are counterstained by safranin.[5,6,9] The results of the Gram stain (e.g., gram-positive cocci in pairs or gram-negative rods) can be used to provide information about the possible infecting organism before the culture results become available. **Table 18-2** lists the most likely bacteria based on Gram stain results.[10,11] In some situations, the clinician can use the information from the Gram stain to select an empiric antibiotic regimen against the most likely pathogen causing the patient's infection before the final culture and susceptibility results are available. Once the culture and susceptibility results are available, the initial empiric antibiotic regimen can be deescalated, if necessary, to target the infecting bacteria (directed therapy).

Besides providing a clue regarding the potential infecting organism, the Gram stain also helps to determine the *presence* of bacteria in biological specimens obtained from normally sterile body fluids (e.g., CSF, pleural fluid, synovial fluid, and urine directly from the bladder) and from specimens where infection is suspected (e.g., abscess fluid, wound swabs, sputum, and tissue); the number or relative quantity of infecting bacteria; the presence of WBCs; and the quality of the submitted specimen (e.g., large numbers of epithelial cells in a sputum or urine sample may signify contamination).[1,4,6,7,9]

Culture and Identification

The results from the Gram stain provide preliminary information regarding the potential infecting bacteria. For the bacteria to be definitively identified, the clinical specimen is also processed to facilitate bacterial growth in culture and then observed for growth characteristics (e.g., type of media, aerobic versus anaerobic, shape and color of colonies) and reactions to biochemical testing. Under normal circumstances, the results of bacterial culture are typically available within 24–48 hours of specimen setup and processing.

For a bacteria to be grown successfully in culture, the specific nutritional and environmental growth requirements of the bacteria must be taken into consideration.[3,4,8,12,13] There are several clinical microbiology textbooks and reference manuals available that can assist the microbiology laboratory with the selection of appropriate culture media and environmental conditions to facilitate the optimal growth of bacteria based on specimen type and suspected bacteria.[3,4,8,12,13]

Several types of primary culture media are available that enhance or optimize bacterial growth including nutritive media (blood or chocolate agar), differential media, selective media, and supplemental broth.[1,3,8,12,13] The most commonly utilized bacterial growth media are listed in **Table 18-3**. Blood and chocolate agar plates are *nutritive* or *enrichment* media because they support the growth of many different types of aerobic and anaerobic bacteria. Blood agar is also considered to be *differential* media because it can distinguish between organisms based on certain growth characteristics, such as the differentiation between streptococci based on hemolysis patterns. MacConkey, eosin methylene blue, colistin nalidixic acid, and phenylethyl alcohol agar plates are considered *selective* media because they preferentially support the growth of specific organisms (e.g., gram-negative or gram-positive bacteria) through the use of antimicrobials, dyes, or alcohol incorporated into their media. Trypticase soy broth and thioglycollate broth are considered *supplemental* media because they are used for subculturing bacteria detected on agar plates, or as back-up cultures to agar plates for the detection of small quantities of bacteria in biological specimens.

Once a clinical specimen is processed on growth media, the plates must be incubated in the appropriate environment to support bacterial growth. The environmental factors that should be controlled during incubation include oxygen or carbon dioxide content, temperature, pH, and moisture content of the medium and atmosphere.[1,12,13] The oxygen requirements for growth differ among organisms. Strict aerobic bacteria, such as *Pseudomonas aeruginosa*, grow best in ambient air containing 21% oxygen and a small amount of carbon dioxide.[1] Strict anaerobes, such as *Bacteroides* spp., are unable to grow in an oxygen-containing environment and require a controlled environment containing 5–10% carbon dioxide for optimal growth. Facultative anaerobes, such as *Escherichia coli* and some streptococci, can grow in the presence or absence of oxygen. Overall, most clinically relevant bacteria grow best at 35–37 °C (the temperature of the human body), a pH of 6.5–7.5, and in an atmosphere rich in moisture, which is the reason why agar plates are sealed (to trap moisture).[12]

Bacteria grown successfully in culture appear as colonies on the agar plates. Bacterial identification is based on the results of

TABLE 18-2. Preliminary Identification of Medically Important Bacteria Using Gram Stain Results[10,11]

GRAM STAIN RESULT	LIKELY BACTERIAL PATHOGEN
Gram-positive (stain purple)	
Gram-positive cocci in clusters	***Staphylococcus* spp.** Coagulase-positive: *S. aureus* Coagulase-negative: *S. epidermidis, S. hominis, S. saprophyticus, S. haemolyticus,* etc.
Gram-positive cocci in pairs	***Streptococcus pneumoniae***
Gram-positive cocci in chains	**Viridans (α-hemolytic) streptococci** (*S. milleri, S. mutans, S. salivarius, S. mitis,* etc.) **Group (β-hemolytic) streptococci** (*S. pyogenes* [group A], *S. agalactiae* [group B], groups C, F, and G streptococci) ***Peptostreptococcus* spp.**
Gram-positive cocci in pairs and chains	***Enterococcus* spp.** (*E. faecalis, E. faecium, E. durans, E. gallinarum, E. avium, E casseliflavus, E. raffinosus*)
Gram-positive bacilli	
Nonspore-forming	***Corynebacterium* spp.** (*C. diphtheriae, C. jeikeium, C. striatum,* etc.) ***Lactobacillus* spp.** ***Listeria monocytogenes*** ***Propionibacterium* spp.** (*P. acnes*)
Spore-forming	***Bacillus* spp**. (*B. anthracis, B. cereus,* etc.) ***Clostridium* spp.** (*C. perfringens, C. difficile, C. tetani*) ***Streptomyces* spp.**
Branching, filamentous	***Actinomyces* spp.** (*A. israelii*) ***Erysipelothrix rhusiopathiae*** ***Nocardia* spp.** (*N. asteroides*)
Gram-negative (stain red)	
Gram-negative cocci	***Neisseria* spp.** (*N. gonorrhoeae, N. meningitidis,* etc.) ***Veillonella* spp.** (*V. parvula*)
Gram-negative coccobacilli	***Haemophilus* spp.** (*H. influenzae, H. parainfluenzae, H. ducreyi,* etc.) ***Moraxella catarrhalis***
Gram-negative bacilli	
Lactose-fermenting	***Aeromonas hydrophila*** ***Citrobacter* spp.** (*C. freundii, C. koseri*) ***Enterobacter* spp.** (*E. cloacae, E. aerogenes*) ***Escherichia coli*** ***Klebsiella pneumoniae*** ***Pasteurella multocida*** ***Vibrio cholerae***
Nonlactose-fermenting	***Acinetobacter* spp.** ***Alcaligenes* spp.** ***Burkholderia cepacia*** ***Morganella morganii*** ***Proteus* spp.** (*P. mirabilis, P. vulgaris*) ***Pseudomonas* spp.** (*P. aeruginosa, P. putida, P. fluorescens*) ***Salmonella* spp.** (*S. typhi, S. paratyphi, S. enteritidis, S. typhimurium*) ***Serratia marcescens*** ***Shigella* spp.** (*S. dysenteriae, S. sonnei*) ***Stenotrophomonas maltophilia***
Other gram-negative bacilli	***Bacteroides* spp.** (*B. fragilis, B. thetaiotaomicron, B. ovatus, B. distasonis*) ***Brucella* spp.** ***Bordetella* spp.** ***Campylobacter jejuni*** ***Francisella tularensis*** ***Helicobacter pylori*** ***Legionella* spp.**
Gram-variable (stain both gram-positive and gram-negative in the same smear)	
Gram-variable bacilli	***Gardnerella vaginalis***

TABLE 18-3. Commonly Used Bacterial Growth Media[1,3,8,12,13]

GROWTH MEDIUM	COMPOSITION	USES
Agars		
SBA	5% sheep blood	The most commonly used all-purpose medium with ability to grow most bacteria, fungi, and some mycobacteria; also used for determination of hemolytic activity of streptococci
Chocolate agar, enriched	2% hemoglobin or Iso-VitaleX in peptone base	All-purpose medium that supports growth of most bacteria; especially useful for growth of fastidious bacteria such as *Haemophilus* spp., *Brucella* spp., and pathogenic *Neisseria* spp.
EMB or Mac agar	Peptone base with sugars and dyes that yield differentiating biochemical characteristics	Included in primary setup of nonsterile specimens; selective isolation of gram-negative bacteria; differentiates between lactose-fermenting and nonlactose-fermenting enteric bacteria
PEA or CNA agar	Nutrient agar bases with supplemental agents to inhibit growth of aerobic gram-negative bacteria	Included in primary setup of nonsterile specimens; selective isolation of gram-positive cocci and bacilli as well as anaerobic gram-positive cocci or gram-negative bacilli
Broths		
TSB	All-purpose enrichment broth	Used for subculturing bacteria from primary agar plates; supports the growth of many fastidious and nonfastidious bacteria
Thioglycollate broth	Pancreatic digest of casein, soy broth, and glucose	Supports the growth of aerobic, anaerobic, microaerophilic, and fastidious bacteria

CNA = colistin-nalidixic acid; EMB = eosin methylene blue; Mac = MacConkey; PEA = phenylethyl alcohol; SBA = sheep blood agar; TSB = trypticase soy broth.

genotypic and phenotypic testing.[12] Genotypic bacterial identification tests employ molecular techniques for the detection of a particular gene or ribonucleic acid (RNA) product that is characteristic of specific bacteria. Phenotypic bacterial identification tests involve the observation of the physical and metabolic properties of a bacteria including the evaluation of colony characteristics (size, pigmentation, shape, and surface appearance); the assessment of culture media and environmental conditions that supported the growth of the bacteria; the changes that occurred to the culture media as a result of bacterial growth; the aroma of the bacteria; the Gram stain result of individual colonies; and the results from biochemical testing.[3,12] Biochemical tests for bacterial identification are either enzyme-based, where the presence of a specific enzyme is measured (e.g., catalase, oxidase, indole, or urease tests), or based on the presence and measurement of metabolic pathways or byproducts (e.g., oxidative and fermentation tests or amino acid degradation).[3,12] Examples of biochemical tests include the presence of catalase in the organism or the ability of a bacteria to ferment glucose. Most biochemical tests are performed using manual or automated commercial (preferred) identification systems.[12,14] Some of the commercial identification systems consist of multicompartment biochemical tests in a single microtiter tray so that several biochemical tests can be performed simultaneously.[12,14] The information derived from the macroscopic examination of the bacteria and the results of biochemical tests are then combined to determine the specific identity of the bacteria. Using traditional methodology, bacterial identification is usually achieved within 24–48 hours of the detection of bacterial growth.[12,13]

Numerous rapid diagnostic tests are now available for the identification of bacteria (with some tests also detecting several pertinent bacterial resistance genes) directly from clinical specimens such as blood, stool, respiratory secretions, or body fluids (i.e., saliva, urine) using a variety of methodologies, which have all been designed to substantially decrease the time to organism identification (with results in 15 minutes to 12 hours) when compared to traditional bacterial culture and identification methods.[3,11,14-16] These rapid diagnostic tests are highly sensitive and specific; they are important tools for optimal antimicrobial stewardship and minimizing the emergence of bacterial resistance because they limit unnecessary antimicrobial therapy (i.e., discontinuation of vancomycin in patients with single blood culture with coagulase-negative staphylococci); and they allow for quicker antimicrobial deescalation and implementation of infection control procedures, such as isolation, when necessary.[3,11,14-16] Rapid diagnostic tests are usually more costly than traditional bacterial identification methods (although studies have demonstrated cost-effectiveness of many of the tests through faster optimization of antimicrobial therapy, improved patient outcomes, and overall lower hospital costs). They are, at this point, only able to detect the presence of limited resistance markers (albeit the most common ones).[3,15,16]

The currently available rapid bacterial identification tests are either immunologically based, nucleic acid (NA)–based (nonamplified and amplified), or proteomic-based.[3,11,14-16] Immunologic methods employ immunofluorescent or enzyme-linked immunosorbent assay (ELISA) antigen or antibody detection, and they are available for the detection of group A *streptococcus* (pharyngeal), *Streptococcus pneumoniae* (urine, respiratory), *Neisseria gonorrhoeae* (urethral, cervical), *Neisseria meningitidis* (cerebrospinal fluid), *L. pneumophila* (urine), influenza/respiratory syncytial virus (RSV) (nasopharyngeal), and other viruses (see respective sections within this chapter for additional information).

A few of the commonly used nonamplified NA–based bacterial identification methods utilizing NA probes include peptide NA fluorescence in situ hybridization (PNA-FISH), QuickFISH (AdvanDx, Woburn, MA), and Verigene (Luminex, Austin, TX).[3,15] PNA-FISH and QuickFISH detect species-specific RNA using fluorescent probes and have been developed in kits to differentiate between commonly encountered bacteria from positive blood cultures after Gram stain (e.g., *Staphylococcus aureus* versus coagulase-negative staphylococci from blood culture bottle with gram-positive cocci in clusters; *Enterococcus faecalis* versus *Enterococcus faecium* versus other enterococci from blood cultures positive for gram-positive cocci in pairs and chains; *E. coli*, *K. pneumoniae* versus *P. aeruginosa* from blood cultures positive for gram-negative rods; *Candida albicans/Candida parapsilosis* versus *Candida krusei/Candida glabrata* from blood cultures positive for yeast). PNA-FISH results are available within 90 minutes, while QuickFISH results are available within 20 minutes of blood culture bottle positivity. An XpressFISH phenotypic test is also available for the detection of the *mecA* gene (a marker for methicillin resistance) in staphylococci, with results available within one hour of specimen setup (performed as a separate test after *S. aureus* has been identified).

Verigene (Luminex, Austin, TX) is a novel microarray, multiplex test utilizing NA extraction and array hybridization in a conserved genetic region of the bacteria or virus to differentiate among different species. Several Verigene tests are currently available for the rapid identification of bacteria and viruses causing infection in the bloodstream, respiratory tract, and GI tract. The Verigene Gram-Positive Blood Culture Test (Luminex, Austin, TX) can identify 12 different gram-positive bacterial species and the presence of three notable gram-positive resistance gene markers, namely *mecA* (methicillin-resistance), *vanA*, and *vanB* (vancomycin resistance) from positive blood cultures within 2.5 hours.[3,15] The Verigene Gram-Negative Blood Culture Test (Luminex, Austin, TX) can identify eight different gram-negative bacteria and the presence of six resistance gene markers (CTX-M β-lactamase and several carbapenemases) from positive blood cultures within two hours. Verigene tests are also available for the rapid identification (within two hours) of the causative organisms of respiratory tract infections, such as the Verigene Respiratory Pathogens Flex Test (Luminex, Austin, TX) with a panel of three bacterial and 13 viral targets); GI infections, such as the Verigene Enteric Pathogens Test (Luminex, Austin, TX), which identifies five bacterial, two viral, and two toxins that cause acute gastroenteritis; and the *C. difficile* test, which can detect the presence of toxin A, toxin B, and PCR ribotype 027 hypervirulent strain).

There are multiple amplified NA–based tests, including PCR and other methods, that are available for the rapid identification of bacteria (and occasionally the presence of resistance gene markers) and viruses. Currently, the most comprehensive test is the FilmArray multiplex PCR system (BioFire Diagnostics, Salt Lake City, UT) that integrates sample preparation, amplification, detection, and analysis in one process with results available within one hour.[3,15] FilmArray is available in several U.S. Food and Drug Administration (FDA)-approved panels including the FilmArray Blood Culture Identification Panel (contains 24 pathogen targets (gram-positive bacteria, gram-negative bacteria and yeast) and three resistance gene targets (*mecA, vanA/B, K. pneumoniae* carbapenemase or KPC; the FilmArray Respiratory Panel (contains 20 bacterial and viral pathogen targets); and the FilmArray Gastrointestinal Panel (contains 22 bacterial, viral, and parasitic targets).

The proteomic-based rapid bacterial identification test is matrix-assisted laser desorption-ionization time-of-flight (MALDI-TOF) mass spectrometry, which is typically applied to colonies grown on an agar plate, although current studies are evaluating the use of this method directly on positive blood cultures.[3,11,14-16] The bacteria are placed on a target plate with a matrix solution and then pulsed with a laser. The ionized particles produced by the laser will differ based on the organism and are identified within 10–30 minutes using a library of standard reference species.[15]

Colonization, Contamination, or Infection

The growth of an organism from a submitted biologic specimen does not always indicate the presence of infection; it may represent the presence of bacterial contamination or *colonization*.[9,11] **Table 18-4** lists the anatomic sites, fluids, and tissues of the human body that are sterile including the bloodstream, the CSF, internal organs and tissues, bone, synovial fluid, peritoneal fluid, pleural fluid, pericardial fluid, and urine directly from the bladder or kidney. Other body sites, particularly those with a connection to the outside environment, are naturally colonized with microorganisms, called *normal flora*. Normal bacterial flora can be found on the skin and in the respiratory, GI, and genitourinary tract (examples of bacteria that typically colonize these body sites are listed in **Table 18-5**).[11,17] Typically, normal flora are harmless bacteria that rarely cause infection. They are often located in the same areas of the body as pathogenic bacteria and are thought to be protective by inhibiting the growth of pathogenic organisms through competition for nutrients and stimulating the production of cross-protective antibodies.[11,17] However, normal flora bacteria may potentially become pathogenic and cause infection in patients with suppressed immune systems or after translocation to normally sterile body sites and tissues

TABLE 18-4. Normally Sterile Body Sites
Bloodstream
CSF
Pericardial fluid
Pleural fluid
Peritoneal fluid
Synovial fluid
Bone
Urine (directly from the bladder or kidney)

CSF = cerebrospinal fluid.

TABLE 18-5. Body Sites with Normal Colonizing Bacterial Flora[11,17]

Skin	**Respiratory tract**
Corynebacterium spp.	Viridans streptococci
Propionibacterium spp.	Anaerobic streptococci
Staphylococcus spp. (especially coagulase-negative staphylococci)	*Haemophilus* spp. *Moraxella* spp.
Streptococcus spp.	*Neisseria* spp.
Gastrointestinal tract	**Genitourinary tract**
Bacteroides spp.	*Lactobacillus* spp.
Clostridium spp.	*Streptococcus* spp.
Escherichia coli	*Staphylococcus* spp.
Klebsiella pneumoniae	*Mycoplasma hominis*
Enterococcus spp.	*Corynebacterium* spp.
Anaerobic streptococci	*Bacteroides* spp.
	Prevotella spp.
	Enterobacteriaceae

TABLE 18-6. Clinical, Laboratory, and Radiographic Signs of Infection

CLINICAL
Localized
Pain and inflammation at site of infection—erythema, swelling, warmth (wound, skin lesion, abscess, cellulitis)
Purulent discharge (wound, vaginal, urethral discharge)
Sputum production and cough (pneumonia)
Diarrhea
Dysuria, frequency, urgency, suprapubic tenderness, costovertebral angle tenderness (UTI)
Headache, stiff neck, photophobia (meningitis)
Systemic
Fever
Chills, rigors
Malaise
Tachycardia
Tachypnea
Hypotension
Mental status changes
LABORATORY
Increased WBC count—peripherally or at the site of infection
Increased neutrophil percentage, including an increase in immature neutrophils (bands or stabs) in the WBC differential called a "shift to the left"
Hypoxemia (pneumonia)
Elevated lactate
Positive Gram stain and culture from site of infection
Elevated ESR and CRP
Elevated procalcitonin levels
Positive antigen test or antibody titers
RADIOGRAPHIC
Chest x-ray with consolidation, infiltrate, effusion, or cavitary nodules in patients with lung infections
Bone x-ray or MRI—periosteal elevation or bone destruction in patients with osteomyelitis
Head CT/MRI—rim-enhancing lesions in patients with brain abscesses

CRP = C-reactive protein; CT = computed tomography; ESR = erythrocyte sedimentation rate; MRI = magnetic resonance imaging; UTI = urinary tract infection; WBC = white blood cell.

during trauma, intravascular line insertion, or surgical procedures, especially when the skin is not adequately cleansed in the latter two situations. In addition, pathogenic bacteria may occasionally *colonize* body sites without invasion of host tissue or elicitation of the signs and symptoms of infection that are listed in **Table 18-6**.

Contamination occurs when an organism is accidentally introduced into a biologic specimen during specimen collection, transport, or processing.[17] Bacteria that cause contamination typically originate from the skin of the patient (especially if the skin is not adequately cleaned before specimen acquisition), the clinician, or the laboratory technician but may also contaminate the specimen from the environment. The most common specimen contaminant overall is coagulase-negative staphylococci (especially *Staphylococcus epidermidis*), which is an organism that normally colonizes the skin.[17] Other common contaminants of blood culture specimens include *Micrococcus* spp., *Propionibacterium acnes*, and most *Bacillus* and *Corynebacterium* spp.[17] In addition, the presence of normal vaginal or perirectal flora in the urine culture of a patient without clinical evidence of a urinary tract infection (UTI), such as symptoms or WBCs in the urinalysis, may also be indicative of contamination.

Infection occurs when an organism invades and damages host tissues eliciting a host response and symptoms consistent with an infectious process. When determining the presence of infection in an individual patient, several factors should be considered such as the clinical condition of the patient (e.g., fever and purulent discharge), the presence of laboratory signs of infection (e.g., high WBC count), the results of microbiologic stains and cultures, and the results from radiographic tests.[9] Table 18-6 describes some of the local and systemic clinical signs and symptoms, laboratory findings, and radiographic findings that may be present in a patient with infection. The exact clinical, laboratory, and radiographic signs of infection are different based on the site of infection, the age of the patient, and the severity of the infection. For example, a patient with pneumonia may exhibit a fever, productive cough, shortness of breath, tachypnea, leukocytosis, and an infiltrate on chest x-ray, and a patient with an uncomplicated UTI will experience urinary frequency, urgency, dysuria, and pyuria. It is important to note that the typical signs and symptoms of infection may not be present in the elderly or in patients who are immunocompromised (e.g., neutropenic patients and patients with acquired immunodeficiency syndrome [AIDS]).

False-positive cultures can lead to the use of additional laboratory tests, radiographic tests, unnecessary antibiotics, increased length of hospitalization, and patient costs; therefore, every positive culture should warrant an evaluation for clinical significance. The diagnosis of infection should be suspected in any patient with a positive culture accompanied by clinical, laboratory, and radiographic findings suggestive of infection. Luckily, certain bacteria have a propensity to cause infection in particular body sites and fluids, as demonstrated in **Table 18-7**.[4,17-19] This information can help determine if the bacteria isolated in a culture is a commonly encountered pathogen at the particular site of infection.[17,18] For instance, the growth of *S. pneumoniae* from the sputum of a patient with signs and symptoms consistent with community-acquired pneumonia (CAP) is a significant finding because *S. pneumoniae* is the most common cause of CAP. However, the growth of *S. epidermidis* from a blood or wound culture from an asymptomatic patient should be evaluated for clinical significance because it may represent contamination of the submitted specimen.[11,17] The information in Table 18-7 regarding the most common causative organisms by infection site can also be used to select empiric antibiotic therapy before culture results are available by guiding the selection of an antibiotic regimen with activity against the most common causative bacteria at the suspected site of infection, as illustrated in **Minicase 1**.

Occasionally, cultures may be negative in patients with infection, particularly in the setting of previous antibiotic use, improper clinical specimen collection methods, or the submission of inadequate specimens. In this setting, the clinical condition of the patient may establish the presence of infection despite negative cultures, and the suspected site of infection should help guide antibiotic therapy based on most likely causative organisms that cause infection at that site.[9]

Antimicrobial Susceptibility Testing

Once an organism has been cultured from a biologic specimen, further testing is performed in the microbiology laboratory to determine the antibiotic susceptibility of the infecting organism to help direct and streamline antimicrobial therapy. Because of the continual emergence of resistance in many organisms, bacterial susceptibility testing is imperative for determining the antimicrobial agents that should be used for the treatment of the patient's infection. There are a number of different methods that can be used to determine the antibiotic susceptibility of a particular organism, and these methods can either (1) directly measure the activity of an antibiotic against the organism, or (2) detect the presence of a specific resistance mechanism in the organism, as described in **Table 18-8**.[9,11,14,20-25] Microbiology laboratories often utilize several different methods for susceptibility testing to accurately determine the activity of antibiotics against many different types of bacteria (e.g., aerobic, anaerobic, and fastidious). The Clinical and Laboratory

TABLE 18-7. Common Pathogens by Site of Infection[4,18,19]

MOUTH	SKIN AND SOFT TISSUE	BONE AND JOINT
Anaerobic streptococci *Peptococcus* spp. *Peptostreptococcus* spp. *Actinomyces israelii*	*Staphylococcus aureus* *Streptococcus pyogenes* *Staphylococcus epidermidis* *Pasteurella multocida* *Clostridium* spp.	*Staphylococcus aureus* *Streptococcus pyogenes* *Streptococcus* spp. *Staphylococcus epidermidis* *Neisseria gonorrhoeae* Gram-negative bacilli
INTRA-ABDOMINAL	**URINARY TRACT**	**UPPER RESPIRATORY TRACT**
Escherichia coli *Proteus mirabilis* *Klebsiella* spp. *Enterococcus* spp. *Bacteroides* spp. *Clostridium* spp.	*Escherichia coli* *Proteus mirabilis* *Klebsiella* spp. *Enterococcus* spp. *Staphylococcus saprophyticus*	*Streptococcus pneumoniae* *Haemophilus influenzae* *Moraxella catarrhalis* *Streptococcus pyogenes*
LOWER RESPIRATORY TRACT COMMUNITY-ACQUIRED	**LOWER RESPIRATORY TRACT HOSPITAL-ACQUIRED**	**MENINGITIS**
Streptococcus pneumoniae *Haemophilus influenzae* *Moraxella catarrhalis* *Klebsiella pneumoniae* *Legionella pneumophila* *Mycoplasma pneumoniae* *Chlamydophila pneumoniae*	**Early onset (within 4 days of hospitalization)** *Klebsiella pneumoniae* *Escherichia coli* *Enterobacter* spp. *Proteus* spp. *Serratia marcescens* *Haemophilus influenzae* *Streptococcus pneumoniae* MSSA **Late onset (>4 days after hospitalization)** Pathogens above plus MDR organisms: *Acinetobacter* spp. *Pseudomonas aeruginosa* *MRSA* *Legionella pneumophila*	*Streptococcus pneumoniae* *Neisseria meningitidis* *Haemophilus influenzae* Group B streptococcus *Escherichia coli* *Listeria monocytogenes*

MDR = multidrug resistant; MRSA = methicillin-resistant *Staphylococcus aureus*; MSSA = methicillin-susceptible *Staphylococcus aureus*.

MINICASE 1

Choosing Empiric Antibiotic Therapy for Hospital-Acquired Pneumonia

Marie A., a 68-year-old female, is admitted to the University Hospital four days ago for management of a right cerebral vascular accident (CVA). Prior to admission, she was living at home with her husband and was previously healthy without recent hospitalizations or antibiotic therapy within the past few years. She initially requires ICU admission for management of her CVA and is transferred to the medical floor after stabilization. She continues to have L-sided hemiparesis and has been deemed to be an aspiration risk by physical therapy/occupational therapy. On hospital day 3, she develops a temperature of 102.3 °F, chills, tachypnea, a productive cough, and shortness of breath requiring supplemental oxygen via nasal cannula.

Her physical exam is significant for an increased RR of 24 breaths/min and decreased breath sounds in the right middle lobe on lung exam. Her laboratory results reveal a total WBC count of 18,000 cells/mm^3 with 70% neutrophils, 19% bands, 7% lymphocytes, and 4% monocytes. Her chest x-ray demonstrates right middle lobe consolidation consistent with pneumonia. Because the differential diagnosis include bacterial pneumonia, an expectorated sputum sample is obtained for Gram stain and culture. The Gram stain reveals >25 WBC/hpf, <10 epi/hpf, and many gram-negative rods. The physician taking care of her asks you to recommend empiric antibiotic therapy to treat her pneumonia before the final culture results are available.

QUESTION: What is the most likely causative organism of this patient's pneumonia, and which empiric antibiotic therapy would you choose based on the Gram stain results?

DISCUSSION: This patient most likely has early onset (within 4 days of hospitalization) hospital-acquired pneumonia (HAP), where the most common causative organisms (Table 18-7) include *S. pneumoniae*; *H. influenzae*; gram-negative bacteria such as *K. pneumoniae*, *E.coli*, *Enterobacter* spp., *S. marcescens*, and *Proteus* spp.; *S. aureus* (methicillin-susceptible *Staphylococcus aureus* [MSSA]); and atypical bacteria such as *L. pneumophila* (especially in patients with diabetes mellitus, underlying lung disease, renal failure, or suppressed immune systems). Based on the Gram stain results demonstrating the presence of gram-negative rods (Table 18-2), the patient most likely has HAP due to *K. pneumoniae*, *E. coli*, *Enterobacter* spp., *S. marcescens*, or *Proteus* spp., which is not unexpected because gram-negative bacteria are the most common cause of HAP overall. Based on the most recent Infectious Diseases Society of America guidelines for the management of HAP, the patient should receive empiric therapy with either ceftriaxone, ertapenem, or a fluoroquinolone (levofloxacin, ciprofloxacin, or moxifloxacin) based on her lack of risk factors for a multidrug-resistant (MDR) organism.[19] The antibiotic regimen can be modified to more directed therapy, if possible, once the results of the culture and susceptibility are available.

TABLE 18-8. Antimicrobial Susceptibility Testing Methods[9,11,14,20-25]

Methods that directly measure antibiotic activity
Dilution susceptibility tests: broth macrodilution (tube dilution), broth microdilution, agar dilution
Disk diffusion: Kirby Bauer
Antibiotic concentration gradient methods: Etest, SGE
Other specialized tests: Measure bactericidal activity—MBC testing, time-kill studies, SBT Susceptibility testing of antibiotic combinations (synergy testing)—checkerboard technique, time-kill curve technique, disk diffusion, Etest
Methods that detect the presence of antibiotic resistance or resistance mechanisms
β-lactamase detection
Detection of HLAR
Agar screens for detection of MRSA or VRE
Chloramphenicol acetyltransferase detection
Molecular methods involving NA hybridization and amplification

Etest = epsilometer test; HLAR = high-level aminoglycoside resistance; MBC = minimum bactericidal concentration; MRSA = methicillin-resistant *Staphylococcus aureus*; NA = nucleic acid; SBT = serum bactericidal test; SGE = spiral gradient endpoint; VRE = vancomycin-resistant enterococci.

Standards Institute (CLSI) continuously updates and publishes standards and guidelines for the susceptibility testing of aerobic and anaerobic bacteria to assist microbiology laboratories in determining the specific antibiotics and test methods that should be used based on the particular organism or the particular clinical situation/infection.[3,20,21,26-28]

Methods That Directly Measure Antibiotic Activity

There are several tests that can measure the activity of an antibiotic against a particular organism. *Quantitative* tests measure the exact concentration of an antibiotic necessary for inhibiting the growth of the bacteria, and *qualitative* tests measure the comparative inhibitory activity of the antibiotic against the organism. The format of the reported test results and interpretation of susceptibility from each of these methods is different depending on the methodology utilized. The advantages and disadvantages of the different antimicrobial susceptibility testing methods are listed in **Table 18-9**.[9,11,20,25,29,30]

Dilution Methods (Macrodilution and Microdilution)

Several dilution methods measure the activity of an antibiotic against a particular organism. Both broth dilution and agar dilution methods quantitatively measure the in vitro activity

TABLE 18-9. Advantages and Disadvantages of Antimicrobial Susceptibility Testing Methods[9,11,20,25,29,30]

METHOD	ADVANTAGES	DISADVANTAGES
Agar dilution	An exact MIC is generated Several isolates can be tested simultaneously on the same plate at a relatively low cost Susceptibility of fastidious bacteria can be determined because agar supports their growth	Very time-consuming Antibiotic plates need to be prepared manually when needed and can be stored only for short periods of time Plates are not commercially available; must be prepared by the laboratory
Broth macrodilution (tube dilution)	An exact MIC is generated The MBC can also be determined, if desired	Each antibiotic is tested individually Method is labor and resource intensive
Broth microdilution (automated)	Simultaneously tests several antibiotics Less labor and resources are used Commercially prepared trays or cards can be used	MIC range (rather than exact MIC) is typically reported The number of antibiotics and concentrations that are tested are predetermined and limited
Disk diffusion (Kirby-Bauer)	Simultaneously tests several antibiotics	Exact MICs cannot be determined Cannot be used for fastidious or slow-growing bacteria
Etest	An exact MIC is generated Easy to perform Several antibiotics can be tested on the same plate	Relatively expensive Not all antibiotics are available as Etest strips
SGE test	An exact MIC is generated Easy to perform	Relatively expensive Can test only one antibiotic at a time Plates are not commercially available; must be prepared by the laboratory

Etest = epsilometer test; MBC = minimum bactericidal concentration; MIC = minimum inhibitory concentration; SGE = spiral gradient endpoint.

of antibiotics against a particular organism. Broth dilution can be performed using *macrodilution* or *microdilution,* where the main differences between the methods include the volume of broth utilized, the number of antibiotics that can be simultaneously tested, and the manner in which the test results are generated and reported. The agar dilution method differs in that it is performed using solid growth media.

Broth macrodilution. Broth macrodilution, or the tube-dilution method, is one of the oldest methods of antimicrobial susceptibility testing and is often considered the gold standard. This method is performed in test tubes in which twofold serial dilutions of the antibiotic being tested for susceptibility (with concentrations used representing clinically achievable serum or site concentrations of the antibiotic in mcg/mL) are placed in a liquid growth media (1 mL of broth or greater) to which a standard inoculum (5×10^5 cfu/mL) of the infecting bacteria is added.[9,20,25-27,29] The tests tubes are incubated for 16–24 hours at 35 °C and then examined macroscopically for the presence of turbidity or cloudiness, which is an indication of bacterial growth.[11,20,25,27,29] The test tube containing the lowest antibiotic concentration that completely inhibits visible growth (the broth in the tube appears clear to the unaided eye) represents the MIC in mcg/mL (**Figure 18-1**).[9,20,25,27,29]

The CLSI has established interpretive criteria for the MIC results of each antibiotic against each bacteria as susceptible (S), intermediate (I), and resistant (R). The exact MICs that separate or define these three categories for an antibiotic are known as their *MIC breakpoints.*[20,21,25] MICs have been categorized as S, I, and R to help predict the probable response of a patient's infection to a particular antibiotic.[9,21,25] Bacteria that are categorized as susceptible to a given antibiotic will, most likely, be eradicated during treatment of the infection because concentrations of the antibiotic represented by the MIC are easily achievable using standard doses of the antibiotic. Intermediately susceptible bacteria display higher MICs, where successful

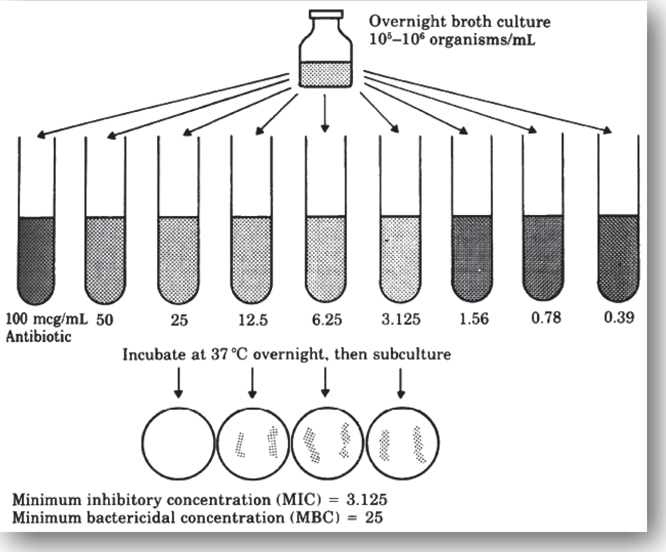

Overnight broth culture
10^5–10^6 organisms/mL
100 mcg/mL Antibiotic
50
25
12.5
6.25
3.125
1.56
0.78
0.39
Incubate at 37 °C overnight, then subculture
Minimum inhibitory concentration (MIC) = 3.125
Minimum bactericidal concentration (MBC) = 25

FIGURE 18-1. Broth macrodilution susceptibility testing for MIC and MBC. (Reprinted, with permission, from reference 9.)

treatment may be achieved if higher than normal doses of an antibiotic are utilized or if the antibiotic concentrates at the site of infection.[25] In clinical practice, antibiotics displaying intermediate susceptibility against an organism are rarely used for treatment of the infection because clinical response is unpredictable. Perhaps one of the only clinical scenarios where intermediately susceptible antibiotics would be used is when the organism displays resistance to all other agents tested. Lastly, organisms that are resistant to an antibiotic display extremely high MICs that exceed the normal achievable serum concentrations of the antibiotic, even if maximal doses are utilized, so that a poor clinical response is expected.

MIC breakpoints for each antibiotic against each bacteria are different because they are based on achievable serum concentrations of the antibiotic after normal dosing; the inherent susceptibility of the organism to the antibiotic; the site of infection and ability of the antibiotic to obtain adequate concentrations at that site; pharmacodynamic analysis with Monte Carlo simulations to predict efficacy; and the results of clinical efficacy trials of the antibiotic in the treatment of infections due to the specific organism with varying MIC values.[20,21,26,27] The safe and effective dose of each antibiotic is typically determined using pharmacokinetic, safety, and efficacy data gathered during preclinical stages of drug development. Because each antibiotic has its own unique pharmacokinetic profile and recommended dosage range, it is not surprising that each antibiotic achieves different serum concentrations after standard dosing. For example, intravenously administered piperacillin/tazobactam achieves higher serum concentrations and area-under-the-serum concentration time curve (AUC) than intravenously administered levofloxacin. Therefore, the MIC breakpoint for susceptibility for piperacillin/tazobactam against Enterobacteriaceae is higher (≤16 mcg/mL) than levofloxacin (≤2 mcg/mL).

Another factor that is considered in the determination of MIC breakpoints is the inherent in vitro activity of the antibiotic against the organism. Some antibiotics are inherently more active against an organism than others (reflected by a lower MIC required to inhibit bacterial growth). The site of infection should also be considered as this may predict the potential usefulness of the antibiotic depending on its ability to achieve adequate concentrations at the site of infection. An antibiotic might be very active against a particular organism in vitro but may be ineffective in vivo due to poor penetration to the site of infection. In fact, there are a few clinical situations where the site of infection is directly incorporated into the MIC susceptibility interpretation of an antibiotic, such as in the case of meningitis due to *S. pneumoniae*, where the interpretation of ceftriaxone and penicillin susceptibility should be determined utilizing meningitis MIC breakpoints of both drugs (which are lower). Monte Carlo analysis using population pharmacokinetic data of the antibiotic and MIC distribution data from susceptibility studies of an organism are also performed in MIC breakpoint determination to evaluate the percentage of time the particular antibiotic being evaluated will achieve adequate serum concentrations or particular pharmacodynamic indices for the treatment of that organism in a simulated population. Lastly, the results from clinical trials evaluating the efficacy of an individual antibiotic are also considered in MIC breakpoint determination where a correlation is made between the individual MIC value of the infecting organism and clinical efficacy or failure (e.g., What was the MIC of the organisms associated with clinical failure of the antibiotic?). In general, it is the responsibility of the clinician to determine if a drug listed as susceptible from an individual isolate susceptibility report is useful for the treatment of a particular infection based on the pharmacokinetic parameters (site penetration) and clinical efficacy studies of the antibiotic for that infection type.

Broth macrodilution is useful because an exact MIC (and, if needed, a minimum bactericidal concentration [MBC] of the infecting organism can be derived. The results of broth macrodilution are reported as the MIC of the antibiotic against the infecting organism with its corresponding interpretive category (S, I, and R). However, broth macrodilution is rarely utilized in microbiology laboratories because the methodology is resource and labor intensive, making it impractical for everyday use.

An additional step can be added to the broth macrodilution test to determine the actual antibiotic concentration that kills 99.9% of the bacterial inoculum, which is also known as the MBC.[20,22,24] Samples from all of the test tubes from the original broth macrodilution test that did not exhibit visible growth are subcultured on agar plates and incubated at 35 °C for 18–24 hours (Figure 18-1).[9,22] The plate representing the lowest antibiotic concentration that does not support the growth of any bacterial colonies is defined as the MBC. Because a higher concentration of an antibiotic may be necessary to kill the organism rather than just inhibit its growth, the MIC is always equal to or lower than the MBC. The determination of the MBC is not routinely performed in clinical practice and is considered only useful in rare clinical circumstances, such as in suspected treatment failure during the treatment of severe or life-threatening infections including endocarditis, meningitis, osteomyelitis, or sepsis in immunocompromised patients.[9,22,24]

Broth microdilution. Broth microdilution susceptibility testing was developed to overcome some of the limitations of the broth macrodilution method and has become the most commonly used method for susceptibility testing of bacteria in microbiology laboratories.[11,20,25,27,29] Instead of utilizing standard test tubes with twofold serial dilutions of antibiotics, this method utilizes manually prepared or commercially prepared disposable microtiter cassettes or trays containing up to 96 wells that can simultaneously test the susceptibility of up to 12 antibiotics depending on the product used.[11,20,25,27,29] Several examples of microtiter trays are shown in **Figure 18-2 (a)**[25] and **Figure 18-2 (b)**. The wells in the broth microdilution trays contain a smaller volume of broth (0.05–0.1 mL) to support bacterial growth than broth macrodilution (1 mL or more). The microtiter tray is inoculated with a standardized inoculum of the infecting organism and incubated for 16–20 hours. The tray is then examined for bacterial growth by direct visualization utilizing light boxes or reflecting mirrors, or by automated, computer-assisted readers. The MIC represents the microdilution well containing the lowest antibiotic concentration that

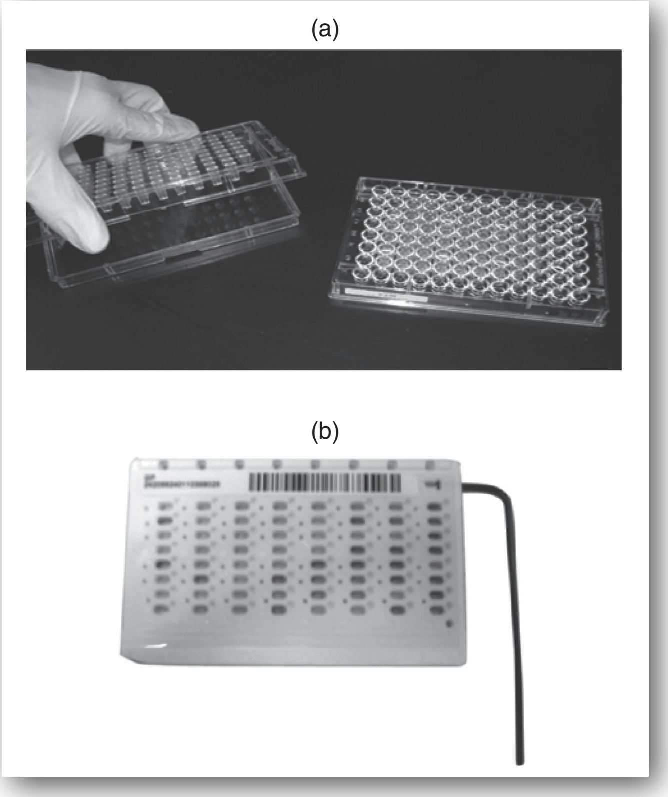


FIGURE 18-2. (a). A broth microdilution susceptibility panel containing 98 reagent wells and a disposable tray inoculator. (Reprinted, with permission, from reference 25.) (b). Example of microtiter cassette used in automated systems that test for bacterial susceptibilities to various antimicrobials. (Reprinted, with permission, from Vitek 2 systems card, bioMérieux, Marcy-l'Étoile, France, 2008.)

completely inhibits visible bacterial growth (e.g., did not produce turbidity). A number of companies commercially supply broth microdilution panels that contain broth with appropriate antibiotic concentrations according to guidelines for conventional broth dilution methods. Depending on the product or system, the results can either be read manually/semiautomated or using automation. Some examples of the manual/semiautomated systems include Sensititre OptiRead or Vizion System (Thermo Fisher Scientific, Waltham, MA) and MicroScan AutoSCAN-4 (Siemens Healthcare Diagnostics, Tarrytown, NY). Examples of the automated systems include Vitek-2 or Vitek-Legacy (bioMérieux Diagnostics, Marcy-l'Étoile, France), MicroScan WalkAway *plus* System (Siemens Healthcare Diagnostics, Tarrytown, NY), Sensititre ARIS 2X (Thermo Fisher Scientific, Waltham, MA), and the Phoenix Automated Microbiology System (BD, Franklin Lakes, NJ).[20] The automated systems also have been engineered to identify bacteria and are able to provide more rapid susceptibility results (within eight hours) due to shortened incubation times.

Because of the size constraints of the broth microdilution panels, only a limited number of antibiotics and concentrations can be incorporated into the trays. Typically, drugs that have inherent activity against the class of bacteria being tested (e.g., gram-positive versus gram-negative) are included in the trays. For example, when determining the susceptibility of Enterobacteriaceae, it is impractical to include antibiotics in the microdilution trays that do not have activity against these organisms, such as penicillin, nafcillin, or vancomycin. The same holds true for susceptibility testing of gram-positive organisms, where it would be impractical to test the susceptibility of piperacillin or ceftazidime against *S. aureus* because these agents have limited antistaphylococcal activity. In addition, the trays are not large enough to incorporate the full range of antibiotic concentrations usually tested using broth macrodilution. Therefore, the concentrations incorporated into the wells for each antibiotic often reflect the CLSI interpretive category breakpoints of S, I, and R for the particular antibiotic-organism combination. Most microbiology laboratories utilize CLSI guidelines and standards to guide appropriate testing and reporting of antimicrobial susceptibilities.

The test results of broth microdilution occasionally include an exact MIC but more often are expressed as an MIC range because of the limited antibiotic concentrations tested for each antibiotic. For example, if bacterial growth is not detected in the lowest concentration tested of a particular antibiotic using broth microdilution, the MIC would be reported as less than or equal to that concentration tested. The MIC could be much lower, but the exact MIC could not be determined because lower concentrations of the antibiotic were not tested due to the size constraints of the microdilution trays. As in broth macrodilution, the test results for broth microdilution are often reported to the clinician as the MIC (or MIC range) of the antibiotic against the infecting bacteria with its corresponding CLSI interpretive category (S, I, and R).

The advantages of broth microdilution include the ability to test the susceptibility of multiple antibiotics simultaneously; the ease of use when commercially prepared microdilution trays are utilized; rapid results with the automated methods; and decreased cost and labor.[20,25,27] The disadvantages of broth microdilution include the lack of flexibility of antibiotics available in commercially prepared microdilution trays; the limitation on the number of concentrations that can be tested for each antibiotic due to size constraints of the trays; and the reporting of an MIC range (on many occasions) rather than the true MIC against an infecting organism.[20,25]

Agar dilution. Agar dilution is another quantitative susceptibility testing method that utilizes twofold serial dilutions of an antibiotic incorporated into agar growth medium, with each concentration placed into individual Petri dishes.[20,25,29] The surface of each plate is inoculated with a droplet of standardized bacterial suspension (1×10^4 cfu/mL) and incubated for 18–20 hours at 35 °C. The susceptibility of several different bacteria can be evaluated simultaneously on the plates. The MIC is represented by the plate with the lowest concentration of antibiotic that does not support visible growth of the bacteria. The advantages of agar dilution include the ability to simultaneously test the susceptibility of a number of different bacteria; the ability to perform susceptibility testing of fastidious organisms because agar is able to adequately support their growth; and the generation of an exact MIC of the infecting bacteria. However, agar dilution is not commonly utilized in most microbiology laboratories because it is resource and labor intensive. In addition, the antibiotic plates are not commercially available and need to be prepared before each susceptibility test because they can be stored only for short periods of time.[20,25,29]

Disk Diffusion Method (Kirby-Bauer)

The *disk diffusion method* is a well-standardized and highly reproducible qualitative method of antimicrobial susceptibility testing that was developed by Kirby and Bauer in 1966, before broth microdilution, in response to the need for a more practical susceptibility test capable of measuring the susceptibility of multiple antibiotics simultaneously.[20,25,28,29] Commercially prepared, filter paper disks containing a fixed concentration of an antibiotic are placed on solid media agar plates inoculated with a standardized inoculum of the infecting organism (1–2 × 10^8 cfu/mL). The plates are large enough to accommodate up to 12 different antibiotic disks at the same time (**Figure 18-3**).[25] The plate is inverted to avoid moisture on the agar surface, and then incubated for 16–18 hours in ambient air at 35 °C. During this incubation time, the antibiotic diffuses out of the disk into the surrounding media, with the highest concentration closest to the disk, as the bacteria multiply on the surface of the plates.[20,25,29] The bacteria will grow only in areas on the plate where the concentrations of the antibiotic are too low to inhibit bacterial growth. At the end of incubation period, the plates are examined for the inhibition of bacterial growth by measuring the diameter (in millimeters) of the clear zone of inhibition surrounding each filter paper disk. In general, the larger the zone size, the more active the antibiotic is against the organism.

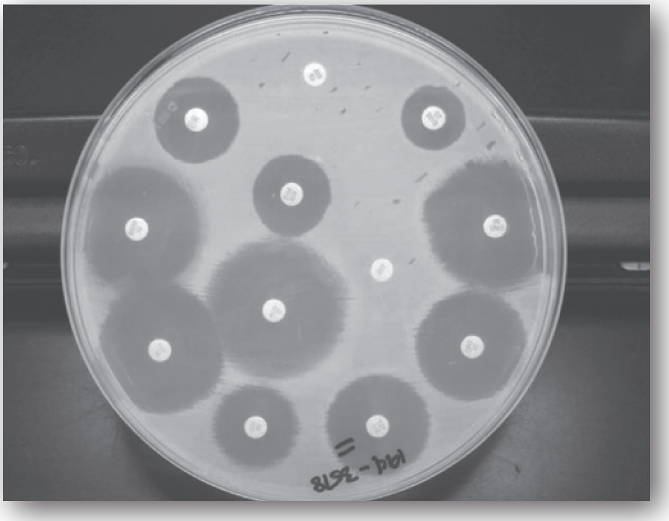


FIGURE 18-3. A disk diffusion test with an isolate of *Escherichia coli* from a urine culture. The diameters of all zones of inhibition are measured and those values translated to categories of susceptible, intermediate, or resistant using the tables published by the CLSI. (Reprinted, with permission, from reference 25.)

The diameter of the zone of inhibition is correlated to the MIC of the antibiotic from broth or agar dilution against the infecting organism using regression analysis.[20,25,28,29] The CLSI has established interpretive criteria based on this relationship to categorize zone diameters as S, I, and R for each antibiotic against each organism.[25,28,29] Subsequently, the results of the disk diffusion test are considered qualitative because the results only reveal the zone of inhibition and category of susceptibility of the antibiotic against the infecting organism rather than an MIC.

The disk diffusion susceptibility test allows the simultaneous testing of a number of antibiotics in a relatively easy and inexpensive manner and also provides flexibility in determining the antibiotics that will be tested for susceptibility, providing a filter paper disk for the desired antibiotic is available. However, the major disadvantages of disk diffusion include the inability to generate an exact MIC and the difficulty in determining the susceptibility of fastidious or slow-growing organisms.

Antibiotic Concentration Gradient Methods

Epsilometer test. The Epsilometer test or Etest (bioMérieux, Marcy-l'Étoile, France) combines the benefits of broth microdilution with the ease of disk diffusion.[11] The Etest method simultaneously evaluates the activity of numerous concentrations of an antibiotic using a single plastic strip impregnated on one side with a known, predefined concentration gradient of an antibiotic. The other side of the Etest strip is marked with a numeric scale that depicts the concentration of antibiotic at that location on the reverse side of the test strip.[9,20,25] Like disk diffusion, the Etest strip is applied onto a solid media agar plate that has been inoculated with a standardized concentration of the infecting bacteria. Several Etest strips can be placed on the same agar plate providing simultaneous susceptibility testing of several antibiotics.[9,20,25] During overnight incubation, bacteria multiply on the agar plates as the antibiotic diffuses out of the Etest strip according to the concentration gradient. Bacterial growth will occur only in areas on the agar plate where drug concentrations are below those required to inhibit growth. An elliptical zone of growth inhibition will form around the Etest strip, and the MIC is read as the drug concentration where the ellipse intersects the plastic strip (**Figure 18-4** and **Figure 18-5**).[20,25]

The results from the Etest are reported as the MIC of the infecting bacteria with the corresponding CLSI susceptibility interpretation. The MIC results derived from the Etest correlate well with the results obtained using other susceptibility testing methods.[9,20,25] The advantages of the Etest method include its ease of use; the ability to evaluate the susceptibility of several antibiotics simultaneously; the exact MIC of the infecting bacteria can be determined; and the laboratory can choose the antibiotics to be tested. However, the Etest method is considerably more expensive than disk diffusion or broth microdilution methods, the results may be reader-dependent, and testing is limited to only those antibiotics for which an Etest strip is commercially available.

The Etest is currently used by some microbiology laboratories for the susceptibility testing of fastidious bacteria, such as *S. pneumoniae, Haemophilus influenzae,* and anaerobes, as well

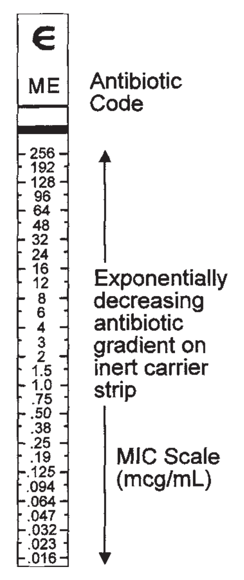


FIGURE 18-4. Diagram of Etest strip and gradient.

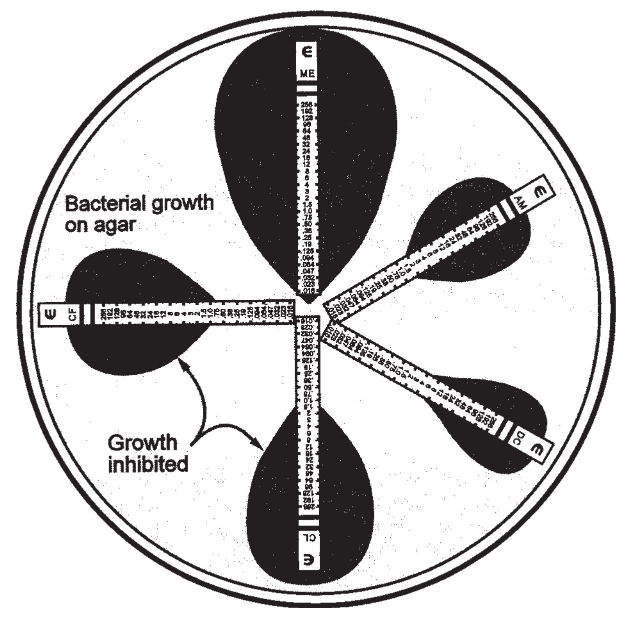


FIGURE 18-5. Etest strips on agar showing inhibition of bacterial growth.

as for those antibiotics in which a routine susceptibility test is not available (i.e., not on standard broth microdilution panels) and when an exact MIC result is preferred.[9,25]

Spiral gradient endpoint test. The spiral gradient endpoint test is performed by using an Autoplate 4000 (Spiral Biotech, Bethesda, MD). This antibiotic gradient diffusion test utilizes agar plates containing a continuous radial concentration gradient of antibiotic in the agar from the center of the plate, where the concentration is the highest, to the edge of the plate, where the concentration is the lowest.[9] The plates are not commercially available but can be made by individual laboratories with specialized equipment. The infecting bacteria is deposited onto the agar as a radial streak and incubated. Up to 15 bacteria can be tested using a single plate. The MIC is determined by measuring the radial distance between growth at the edge of the plate and where growth is inhibited toward the center of the plate. This measurement is used to compute the concentration of antibiotic at that particular location, which is the MIC.[9]

This method is relatively easy to perform and generates an exact MIC of the antibiotic against the infecting bacteria. However, it is relatively expensive, can test only the susceptibility of one antibiotic per plate, and requires the use of specialized equipment. Therefore, it is not routinely used by most microbiology laboratories.

Specialized Susceptibility Tests

Additional tests may be performed in the microbiology laboratory to provide further information on the activity of an antibiotic against an organism. These specialty susceptibility tests may measure the bactericidal activity of the antibiotic (e.g., MBC testing, time-kill curves, and serum bactericidal tests [SBTs]), or the activity of antimicrobials in combination against an infecting organism (e.g., synergy testing using the checkerboard technique or time-kill studies). These tests are not routinely performed in most microbiology laboratories due to both biological and technical difficulties, complexity in the interpretation of the results, and uncertain clinical applicability.[22-24,31]

Testing methods for determining bactericidal activity. Several methods measure the direct killing activity of an antibiotic against an organism and, if used, should be performed only for antibiotics that are generally considered to be bactericidal. As noted earlier, there are only a limited number of clinical circumstances where this information may be useful. The determination of bactericidal activity may have the best clinical utility in the treatment of infections at anatomic sites where host defenses are minimal or absent such as endocarditis, meningitis, and osteomyelitis; as well as in the treatment of severe and life-threatening infections in immunocompromised patients.[9,20,22-24,31] Testing methods that determine the bactericidal activity of an antibiotic include the MBC test, time-kill assays, and SBTs.[20,22-24,31]

The MBC is the lowest concentration of an antibacterial agent that kills 99.9% of the bacterial inoculum, which represents a ≥3 log reduction in the original inoculum.[11,23] The methodology for determination of the MBC has been previously described in detail in the section on broth macrodilution because it is an extension of that test. CLSI has developed guidelines to standardize the methodology for MBC testing.[31] If the MBC exceeds the achievable serum concentrations of the antibiotic, "tolerance" or treatment failure may be observed.[20] Tolerance occurs when a normally bactericidal antibiotic only can inhibit the growth of bacteria based on MBC testing. Minimum bactericidal concentration testing is not routinely performed by most laboratories because it is labor intensive with limited clinical utility.[20,22,31]

Time-kill studies, also known as *time-kill curves,* measure the rate of bacterial killing over a specified period of time, which is unlike the MBC that only measures the bactericidal activity of the antibiotic at a single point in time following an incubation period.[20,24,31] For time-kill studies, a standardized bacterial inoculum is inserted into test tubes containing broth with several different concentrations of an antibiotic (usually the MIC and multiples of the MIC in separate tubes). Samples of the antibiotic-broth solutions are obtained at predetermined time intervals to evaluate the number of viable bacterial colonies present over the 24-hour incubation period.[11,20,24,31] The number of viable bacteria present at each time point are plotted over time to determine the rate and extent of bacterial killing of the antibiotic against the organism. A ≥3 log reduction in viable bacterial counts is representative of bactericidal activity.[11,20,24,31] Because of the labor and resources involved, this test is not routinely performed in many clinical microbiology laboratories, but it is often used in the research setting.

The SBT or Schlichter's test is similar to MIC and MBC testing, except the SBT testing method measures the bacterial killing activity of the patient's serum against his or her own infecting organism after receiving a dose of an antibiotic.[9,20,23,24,31-33] The methodology is very similar to determining the MIC using broth macrodilution, but dilutions of the patient's serum are utilized instead of twofold serial dilutions of an antibiotic.[9,24,31-33] The patient's serum is obtained at predefined intervals before and after a dose of an antibiotic, specifically at the time of expected peak concentration and at the time of expected trough concentration. The patient's serum is then serially diluted and inoculated with a standardized concentration of the infecting organism. The SBT is the highest dilution of the patient's serum that reduces the original standardized bacterial inoculum by ≥99.9%. The results of the SBT are reported as a titer, which represents the number of twofold serial dilutions of the patient's serum that led to bacterial killing (e.g., SBT = 1:16), with a higher titer indicating better activity against the organism.[20,22,24,31-33] The CLSI has developed methodology standards for performance of the SBT.[31,33] However, this test is not routinely performed by most microbiology laboratories due to technical difficulties. Limited data have been published regarding the clinical usefulness of SBTs in guiding therapy (only a few studies in the treatment of endocarditis, osteomyelitis, and serious infections in febrile neutropenia).[22-24,31-33]

Antimicrobial combination testing (synergy testing). In the treatment of bacterial infections, there are several clinical situations where combination antimicrobial therapy will be utilized. The decision to use combination therapy is primarily based on the severity of infection, the causative organism, and the particular type of infection.[11] The potential benefits of combination antibiotic therapy include (1) expanding the antimicrobial spectrum of activity, especially for empiric therapy for a life-threatening infection or for the treatment of polymicrobial infections; (2) producing synergistic bactericidal activity with the combination that is not achieved with each agent alone, such as the use of ampicillin and gentamicin for the treatment of Enterococcal endocarditis; and (3) decreasing the emergence of resistant organisms, which has most notably been observed in the treatment of tuberculosis (TB).[24] Routine antimicrobial susceptibility tests measure the activity of one antibiotic against a particular organism. There are several tests, however, that evaluate the effects of combination antimicrobial therapy against an infecting organism, with the results being expressed as one of three types of activity[11,20]:

1. **Synergy:** The activity of the antimicrobial agents in combination is significantly greater than the additive effects of each agent alone.
2. **Indifference:** The activity of the antimicrobial agents in combination is similar to the additive effects of each agent alone.
3. **Antagonism:** The activity of the antimicrobial agents in combination is less than the additive effects of each agent alone.

Therefore, before two antibiotics are utilized together, it may be useful to determine the effects of the combined antibiotics against the infecting organism, especially because some antibacterial combinations may produce suboptimal effects.

Synergy testing of an antimicrobial combination can be performed using the checkerboard technique, the time-kill curve technique, the disk diffusion assay, or the Etest method.[11,20,24] The checkerboard and time-kill curve techniques are the tests used most often. The checkerboard technique is performed in macrodilution tubes or microdilution plates containing serial dilutions of the antibiotics alone and in combination. The tubes or plates are incubated with a standardized inoculum of the infecting bacteria for 24 hours. The effect of the antibiotic combination is determined by comparing the MICs of the agents when used in combination with the MICs of each agent alone. A synergistic combination displays lower MICs than when each agent is used alone. The time-kill curve method for combination therapy is similar to the time-kill curve method used to determine the rate of bacterial killing of a single agent, except that two antibiotics are added to the tubes in fixed concentrations. The effect of the antibiotic combination is determined by comparing the time-kill rates of combination therapy with the time-kill rates of each agent alone. A synergistic combination displays 100-fold or more killing activity than the most potent agent tested alone.[11,20] In the clinical setting, synergy testing methods are not routinely performed due to their tedious, time-consuming methodologies; their expense; and their limited clinical applicability in predicting clinical outcome.[11,20,24]

Methods Detecting the Presence of Antibiotic Resistance Mechanisms

Detection of β-Lactamase Activity

To date, over 1,300 different β-lactamase enzymes have been characterized.[34] β-lactamase enzymes can be chromosomally, plasmid, or transposon-mediated and may be produced constitutively or inducibly. These enzymes cause hydrolysis of the cyclic amide bond in the β-lactam ring and, depending on the type of enzyme, may result in inactivation of one or numerous β-lactam antibiotics. It is important to understand the consequences of detecting a particular β-lactamase enzyme in an organism because certain enzymes produce resistance only to certain antimicrobials.[20,23,34,35]

A number of methods detect the presence of a β-lactamase enzyme depending on the organism and type of β-lactamase enzyme suspected. Some tests directly detect the presence of β-lactamase activity, while other β-lactamase enzymes (such as the inducible or extended-spectrum β-lactamases [ESBLs]) can be suspected based on resistance patterns and MICs derived from routine susceptibility tests.

The assays that directly detect β-lactamase activity include the acidimetric, iodometric, and chromogenic tests. All of these tests directly measure the presence of β-lactamase enzyme by observing a color change based on reactions to different substrates and can be performed in a short period of time with results available within minutes to hours.[23] The chromogenic test is the most common direct test utilized by microbiology laboratories due to its reliability in detecting β-lactamase enzymes produced by many different bacteria.[23] The chromogenic tests utilize chromogenic cephalosporins (nitrocefin, cefesone, or cefinase) incorporated into filter paper disks or strips that produce a colorimetric change when hydrolyzed by β-lactamase enzymes present in a clinical specimen that is inoculated onto the disk or strip. Test tube assays utilizing chromogenic cephalosporins can also be also utilized. A positive reaction using one of these direct β-lactamase tests for *H. influenzae, Moraxella catarrhalis,* and *N. gonorrhoeae* predicts resistance to only penicillin, ampicillin, and amoxicillin but not to other β-lactam antibiotics that are more stable to hydrolysis by β-lactamase enzymes. A positive β-lactamase test for *Staphylococcus* spp. predicts resistance to penicillin, ampicillin, amoxicillin, carbenicillin, ticarcillin, and piperacillin.

ESBLs are plasmid-encoded β-lactamase enzymes that can be inhibited by β-lactamase-inhibitors, such as clavulanic acid, and can hydrolyze penicillins; first-, second-, and third-generation cephalosporins; and aztreonam.[35] For some organisms, such as some Enterobacteriaceae and *Pseudomonas* spp., the production of ESBL enzyme may be inducible so that the detection of β-lactamase enzyme cannot fully predict the antibiotic susceptibility (or resistance) of the organism.[23,36] Therefore, direct β-lactamase testing for these organisms is not recommended because it may produce misleading results. In the recent past, routine susceptibility tests using CLSI breakpoints did not always detect ESBL-producing organisms. The detection of ESBLs in these organisms has improved with the introduction of CLSI guidelines outlining the use of confirmatory tests involving MIC and disk diffusion screening breakpoints for particular antibiotics using β-lactamase inhibitors.[21,23,26-28,34-37] In addition, several automated systems such as Vitek 2 and the Phoenix System contain ESBL detection tests that, when used with expert system software, are able to accurately detect ESBLs, including some ESBLs not detected by the CLSI confirmatory methods.[37]

AmpC β-lactamases are chromosomally or plasmid-mediated β-lactamase enzymes that hydrolyze first-, second-, and third-generation cephalosporins and cephamycins, and they also display resistance to currently available β-lactamase-inhibitors such as clavulanic acid, sulbactam, and tazobactam. Many gram-negative bacteria, such as ***S.*** *marcescens,* ***P.*** *aeruginosa,* **i**ndole-positive *Proteus* spp., ***A****cinetobacter* spp., ***C****itrobacter freundii*, and ***E****nterobacter* spp. (often referred to as the *SPICE* or *SPACE* bacteria) contain chromosomally mediated, inducible AmpC enzymes that, when hyperproduced, can also hydrolyze penicillins and aztreonam in addition to the cephalosporins and cephamycins listed above.[37] AmpC hyperproduction can occur during the treatment of infection due to one of these organisms, especially when a strong inducer is used such as ceftazidime or clavulanic acid.[35] Plasmid-mediated AmpC enzymes have been reported in *Klebsiella* spp., *Proteus mirabilis, Citrobacter koseri,* and *Salmonella* spp., and often display an antibiotic susceptibility profile similar to that of chromosomally mediated AmpC hyperproducers.[37] All SPICE and SPACE bacteria should be assumed to be AmpC producers, so that specific tests to detect AmpC production are not essential.[37] Plasmid-mediated AmpC β-lactamases can be detected by demonstrating cephamycin hydrolysis using the AmpC disk test, the modified Hodge test (MHT), or the three-dimensional test.[37]

Several types of carbapenemase enzymes have been characterized (metallo-β-lactamases, serine carbapenemases such as *K. pneumoniae* carbapenemase or KPC), which are able to hydrolyze carbapenems and other β-lactam antibiotics.[34,37] Carbapenemase enzymes may be chromosomally (*Stenotrophomonas maltophilia*) or plasmid-mediated (KPCs, *P. aeruginosa, Acinetobacter* spp.), with plasmid-mediated strains often displaying resistance to multiple other drug classes.[37] The MHT can be used for carbapenemase detection on isolates with elevated carbapenem MICs; however, it cannot differentiate between carbapenemase types.[23,37]

In 2010, the CLSI lowered the cephalosporin and carbapenem breakpoints for Enterobacteriaceae in an attempt to better identify antibiotic agents with predictable efficacy against bacteria with multiple resistance mechanisms and eliminated the recommendation to perform specialized testing to detect ESBL-, AmpC-, or carbapenemase-mediated resistance. However, this recommendation has gained considerable criticism from many clinicians and microbiologists because detection of the exact mechanism of resistance is thought to be important for both treatment and epidemiologic purposes.[37]

High-Level Aminoglycoside Resistance

The aminoglycoside antibiotics have relatively poor activity against *Enterococcus* spp. due to poor intracellular uptake, so they should not be utilized as monotherapy in the treatment of infections due to enterococci. However, they may be combined with ampicillin, penicillin, or vancomycin to provide synergistic bactericidal activity, which may be necessary for the treatment of Enterococcal endocarditis or Enterococcal osteomyelitis. Gentamicin and streptomycin are the most commonly used aminoglycosides in this situation and are, therefore, the agents most commonly tested for synergistic activity. Supplemental testing can be performed to detect the presence of high-level aminoglycoside resistance (HLAR), which predicts the lack of synergism between gentamicin or streptomycin and cell-wall active agents against *Enterococcus* spp.[23,36]

The presence of HLAR can be evaluated using the agar dilution screening method utilizing agar plates containing high concentrations of gentamicin (500 mcg/mL) and streptomycin (2000 mcg/mL) or the broth microdilution method where the well contains brain heart infusion (BHI) broth containing high concentrations of gentamicin (500 mcg/mL) and streptomycin (1000 mcg/mL).[23] The plates or wells are inoculated with a standardized suspension of the infecting *Enterococcus* spp. and incubated for 24 hours in ambient air.[23] The growth of one or more *Enterococcus* spp. colonies on the agar plate or in the broth microdilution well demonstrates the presence of HLAR, signifying that the corresponding aminoglycoside cannot be used with a cell-wall active agent to achieve synergistic bactericidal activity. HLAR can also be detected utilizing a disk diffusion method where disks containing high concentrations of gentamicin (120 mcg) and streptomycin (300 mcg) are utilized.[23] HLAR to gentamicin also confers resistance to tobramycin, netilmicin, and amikacin but not necessarily streptomycin, which should be tested independently.[23] A modified test using kanamycin may be used to predict HLAR to amikacin for strains of *E. faecalis;* however, this test is not generally available in most laboratories.[23] Testing for HLAR is usually performed only on Enterococcal isolates from infections that require combination bactericidal activity, such as bacteremia, endocarditis, osteomyelitis, or meningitis.[23]

Tests for the Detection of MRSA, VISA, VRSA, and VRE

Several tests are available that can quickly detect or confirm the presence of methicillin-resistant *S. aureus* (MRSA) or vancomycin-resistant enterococci (VRE). For the detection or confirmation of MRSA, the cefoxitin disk diffusion test, oxacillin–salt agar screening tests, culture-based chromogenic media, rapid latex agglutination (LA) tests, or molecular methods utilizing real-time polymerase chain reaction (PCR) can be utilized.[23,30,38-41]

The cefoxitin disk diffusion test is performed using routine CLSI procedures, with modified interpretive criteria utilized to detect MRSA, where MRSA is reported for *S. aureus* strains with a zone size of ≤21 mm.[23,28] This test has also been useful in detecting methicillin-resistance in coagulase-negative staphylococci.[23] The oxacillin–salt agar screening tests have been widely used for the detection of MRSA, but they appear to lack sensitivity for the detection of strains that exhibit heteroresistance.[23] A standard inoculum of *S. aureus* is inoculated onto an agar plate containing Mueller-Hinton agar supplemented with 4% sodium chloride and 6 mcg/mL of oxacillin and incubated in ambient air for 24 hours.[23,36] The growth of more than one colony indicates MRSA, which also confers resistance to nafcillin, oxacillin, cloxacillin, dicloxacillin, and all cephalosporins excluding ceftaroline. However, this test is not recommended for the detection of methicillin-resistance in other *Staphylococcus* spp.[23,36]

Culture-based chromogenic media—some of which include MRSASelect (Bio-Rad Laboratories, Redmond, WA), Spectra MRSA (Remel Laboratories, Lenexa, KS), CHROMagar MRSA II (BD, Sparks, MD), ChromID MRSA (bioMérieux, Marcy-l'Étoile, France), and HardyCHROM MRSA (Hardy Diagnostics, Santa Maria, CA)—produce a characteristic pigment in the presence of MRSA.[23] These tests are typically utilized for MRSA screening rather than for the diagnosis of infection and are less sensitive and slower than molecular methods.[23] There are numerous rapid commercial LA tests for the detection of MRSA—including MRSA Screen Test (Denka-Seikin, Tokyo, Japan), the PBP 2' Test (Oxoid Limited, Basingstroke, UK), the Mastalex Test (Mast Diagnostics, Bootle, UK), and the Slidex MRSA Detection test (bioMérieux, Marcy-l'Étoile, France)—that employ LA using highly specific monoclonal antibodies for the detection of penicillin-binding protein (PBP) 2a (also termed *PBP 2`),* the protein encoded by the *mecA* gene in MRSA.[23,40] These tests can be performed within 10–15 minutes from bacterial colonies cultured on blood agar plates, decreasing the overall MRSA detection time by approximately 24 hours when compared to standard methods, and are highly sensitive and specific for the detection of MRSA.[40]

The GeneOhm MRSA Assay (BD, Franklin Lakes, NJ), the Xpert MRSA (Cepheid, Sunnyvale, CA), and the LightCycler MRSA Advanced test (Roche Diagnostics, Indianapolis, IN) are approved real-time PCR assays for the rapid, direct detection of nasal colonization by MRSA for the prevention and control of MRSA infection in healthcare institutions.[23,30,38,39] These assays can detect the presence of MRSA directly from nasal swab specimens within two hours using real-time PCR that couples primers specific for *mecA* and the *S. aureus*-specific gene *orfX* (sensitivity 93%, specificity 96%).[38,39] Several PCR-based tests also exists for the detection of MRSA from blood cultures positive for gram-positive cocci in clusters such as XPert MRSA/SA BC test, (Cepheid, Sunnyvale, CA), and FilmArray (BioFire Diagnostics, Salt Lake City, UT), with results typically available within one hour of culture positivity.[41]

The CLSI reference broth microdilution method can accurately detect vancomycin intermediate *S. aureus* (VISA, MIC 4–8 mcg/mL) and vancomycin-resistant *S. aureus* (VRSA, MIC ≥16 mcg/mL) but may not consistently detect the presence of heteroresistant VISA (hVISA).[23] The use of BHI plates with 6 mcg/mL of vancomycin (VRE screening plates described below) can be considered for the detection of *S. aureus* strains with an MIC of 8 mcg/mL but is not useful for VISA strains with an MIC of 4 mcg/mL.[11,23,36] Lastly, the disk diffusion test is unable to accurately detect VISA strains but will detect VRSA strains mediated by *vanA*.[23]

Current automated susceptibility testing methods, including Vitek 2 and the Phoenix system, are now able to accurately detect the presence of VRE.[23] VRE can also be detected utilizing the vancomycin agar screen test, and is often performed on rectal swab specimens to detect carriers of VRE. A standard inoculum of the infecting *Enterococcus* spp. is inoculated onto an agar plate supplemented with BHI broth containing vancomycin 6 mcg/mL and incubated in ambient air for 24 hours.[23,36]

The presence of any growth demonstrates the presence of VRE. This test is most useful for detecting acquired vancomycin resistance in *E. faecalis* and *E. faecium,* but it is not as useful for strains that display intrinsic resistance to vancomycin, such as *E. gallinarum* and *E. casseliflavus* where MICs range from 2–32 mcg/mL and growth is variable on agar screening plates.

D Test for Detecting Inducible Clindamycin Resistance

Resistance to clindamycin in staphylococci and β-hemolytic streptococci is typically mediated by expression of the *erm* gene, which confers resistance to macrolides, lincosamides, streptogramin b, or MLSb-type resistance, and which can be constitutive or inducible.[20,23] Staphylococcal or β-hemolytic streptococcal isolates that are macrolide resistant but clindamycin susceptible should be evaluated for inducible clindamycin resistance using the D test.[20,23] The D test is a disk diffusion procedure where a 15-mcg erythromycin disk is placed 15–26 mm apart from a 2-mcg clindamycin disk on an agar plate inoculated with the infecting organism.[20,23] If inducible clindamycin resistance is present in the organism, the clindamycin zone of inhibition will be flattened on the side nearest the erythromycin disk, demonstrating the letter *D* in appearance. Organisms that display a flattening of the clindamycin zone are D-test positive and should be reported resistant to clindamycin in the final organism susceptibility report.

Special Considerations for Fastidious or Anaerobic Bacteria

The susceptibility testing of fastidious bacteria (e.g., *H. influenzae, N. gonorrhoeae,* and *S. pneumoniae*) and anaerobes cannot be performed utilizing standard broth microdilution, disk diffusion, or automated susceptibility testing methods because these organisms require more complex growth media and environmental conditions to support bacterial growth.[36,42-45] The cultivation of fastidious bacteria or anaerobes may require media with supplemental nutrients, prolonged incubation times, and incubation in atmospheres with higher CO_2 concentrations.[45] Microbiology reference texts and CLSI standards have been developed to outline specific methodologies (broth dilution, disk diffusion, and automated methods), quality control guidelines, and interpretive breakpoint criteria that should be utilized for the susceptibility testing of these bacteria.[21,25-29,36,42-45]

The clinical significance of anaerobes as a cause of infection is more widely appreciated, and the susceptibility of anaerobes to various anti-infective agents is no longer predictable.[9,42-44,46,47] The handling and processing of biologic specimens for anaerobic culture and susceptibility testing are extremely crucial to the validity of the results because most anaerobic bacteria of clinical importance are intolerant to oxygen.[9,42] Specimens should be collected in appropriate anaerobic transport systems (commercially available vials or tubes) that contain specialized media and atmospheric conditions to support the growth of the anaerobic bacteria until the specimen is processed in the laboratory.[42] Once collected, the specimens should be transported to the laboratory within minutes to hours of collection, setup for culture in anaerobic jars or chambers in the appropriate growth media, and incubated in anaerobic atmospheric conditions. The clinical specimens that provide the best yield for anaerobic culture include aspirated or tissue biopsy specimens.[42]

The identification of anaerobic bacteria by an individual hospital laboratory may be performed using one of three methods: (1) presumptive identification based on information from the primary growth plates including the Gram stain results, patterns of growth on selective or differential media, plate and cell morphology, and results of various rapid spot and disk tests; (2) definitive identification based on the results of individual biochemical tests that detect the presence of preformed enzymes found in certain anaerobes; and (3) rapid identification of anaerobes using commercially available detection panels, which also rapidly detect the presence of preformed enzymes such as the BBL Crystal Anaerobe ID (BD, Franklin Lakes, NJ), the RapID-ANA II System (Thermo Fisher Scientific, Waltham, MA), the Rapid Anaerobe Identification Panel (Dade Microscan, West Sacramento, CA), or the AnIdent and Vitek 2 ANC card (bioMérieux, Marcy-l'Étoile, France).[42] Many hospital laboratories do not have the resources for commercially available, anaerobic bacteria identification systems and rely on the first two methods for presumptive identification of anaerobic bacteria. If necessary, clinical isolates can be sent to a reference laboratory for further testing.

Most clinical microbiology laboratories do not currently offer routine susceptibility testing of anaerobic bacteria because of the uncommon occurrence of pure anaerobic infections, the uncertain role of anaerobes in mixed infections, the previous predictable susceptibility of anaerobic bacteria to antibiotics, the previous lack of standardization of antimicrobial susceptibility testing of anaerobes, and the technical difficulties in performing the tests.[42,44,46] However, it is becoming apparent that routine antimicrobial susceptibility testing of anaerobic bacteria is necessary due to the increasing incidence of serious infections caused by anaerobic bacteria, the emerging resistance of anaerobic bacteria to multiple antibiotic agents, and the poor clinical outcomes observed when ineffective antibiotics are utilized for the treatment of infections due to anaerobes.[42,44,46,47]

The susceptibility testing of anaerobic bacteria has undergone numerous methodological modifications and standardization over the past several years.[9,44, 46,47] The CLSI has recently published a standard outlining the clinical situations where anaerobic susceptibility testing should be considered, the methods of susceptibility testing that should be utilized, when and how surveillance susceptibility reporting should be performed, and the antibiotic agents that should be tested for susceptibility.[46]

Susceptibility testing for anaerobes should be performed in patients with serious or life-threatening infections such as endocarditis, brain abscess, osteomyelitis, joint infection, refractory or recurrent bacteremia, and infection of prosthetic devices or vascular graft infections.[9,42,44,46] Susceptibility testing should also be performed in patients with persistent or recurring anaerobic infections despite appropriate antibiotic therapy.[9,44,46] Lastly, susceptibility testing of anaerobic bacteria should be periodically performed within geographic areas or individual institutions to monitor regional susceptibility patterns of anaerobic bacteria over time.[42,44,46]

The recommended anaerobic susceptibility testing methods include agar dilution and broth microdilution using supplemented *Brucella* broth, both of which can be reliably performed by most clinical microbiology laboratories.[9,42,44,46,47] The agar dilution method is the gold standard reference method that can be utilized for susceptibility testing of any anaerobic bacteria, while the broth microdilution method has been validated only for antimicrobial susceptibility testing of *Bacteroides fragilis* group organisms.[44,46] In contrast to agar dilution, the broth microdilution method can evaluate the susceptibility of multiple antibiotics simultaneously, and several microdilution panels are now commercially available for routine testing including Anaerobe Sensititre panel (ANO2, Thermo Fisher Scientific, Waltham, MA) and Oxoid ANA MIC Panel (Thermo Fisher Scientific, Waltham, MA). The general methodology for each of these tests is similar to those described above for aerobic bacteria. In addition, Etest strips can also be used for anaerobe susceptibility testing, and results appear to correlate well with agar dilution. Broth disk elution and disk diffusion are not recommended because their results do not correlate with the agar dilution reference method.[42,44,46,47] β-lactamase testing of anaerobes can be performed according to CLSI guidelines using chromogenic disks.[44,46,47]

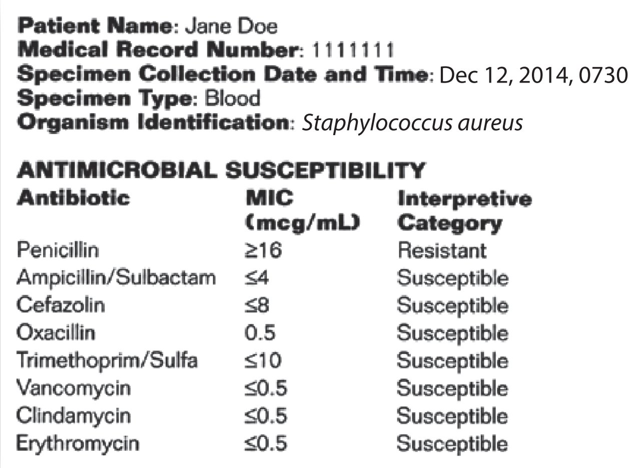
Because routine antimicrobial susceptibility of anaerobes is not performed by all hospital microbiology laboratories or for all anaerobic isolates, antibiotic therapy for infections due to anaerobes is usually selected empirically based on susceptibility reports published by reference laboratories.[44] However, if susceptibility testing is performed on an individual anaerobic isolate, the results should be used to guide the anti-infective therapy for the patient.

Methods for Reporting Susceptibility Results

Individual Isolate Susceptibility Reports

When a bacterial isolate is recovered from a clinical specimen, the identification and susceptibility results are compiled in a report that is available electronically or via a hard copy in the patient's chart. The bacterial identification and antibiotic susceptibility report often contains the following information: the patient's name, medical record number, the date and time of specimen collection, the source of specimen (e.g., blood, wound, urine, etc.), the bacteria that were identified (if any), and the list of antibiotics tested for susceptibility along with the patient's MIC or disk diffusion results and CLSI interpretive category, as shown in **Figure 18-6**.[20,48] In some hospitals, the individual isolate susceptibility report may also contain information regarding the usual daily doses and costs of antibiotics.

Once the bacterial culture and susceptibility results are available, this information should be utilized to change the patient's empiric antibiotic regimen, which usually covers a broad spectrum of bacteria, to a more *directed* antibiotic regimen targeting the infecting bacteria and antibiotic susceptibility. The directed antibiotic regimen should be chosen based on clinical and economic factors, some of which include the severity of infection, the site of infection, the activity (MIC value) of the antibiotic against the infecting organism, the proven efficacy of the antibiotic in the treatment of the particular infection, the overall spectrum of activity of the antibiotic (a narrow spectrum agent is preferred), the end-organ function of the patient, the presence of drug allergies, the route of administration (oral versus parenteral), the daily cost of the antibiotic, etc. The susceptibility report provides some of the information necessary for the deescalation of antibiotic therapy, namely, the site of infection, the identification of the infecting organism(s), and the susceptibility of the infecting organism(s).



Patient Name: Jane Doe
Medical Record Number: 1111111
Specimen Collection Date and Time: Dec 12, 2014, 0730
Specimen Type: Blood
Organism Identification: *Staphylococcus aureus*

ANTIMICROBIAL SUSCEPTIBILITY

Antibiotic	**MIC (mcg/mL)**	**Interpretive Category**
Penicillin	≥16	Resistant
Ampicillin/Sulbactam	≤4	Susceptible
Cefazolin	≤8	Susceptible
Oxacillin	0.5	Susceptible
Trimethoprim/Sulfa	≤10	Susceptible
Vancomycin	≤0.5	Susceptible
Clindamycin	≤0.5	Susceptible
Erythromycin	≤0.5	Susceptible

FIGURE 18-6. Example of microbiology laboratory report with bacterial identification and antibiotic susceptibility.

As seen in the sample susceptibility report in Figure 18-6, there may be a number of antibiotics to which the infecting bacteria is susceptible, often with differing MICs. It is not always advantageous to choose the antibiotic with the lowest MIC against a particular organism on a susceptibility report. As discussed earlier in this chapter, antibiotics have different MIC breakpoints corresponding to S, I, and R for each bacteria based on a number of factors. Some drugs, such as the parenteral piperacillin/tazobactam, are assigned higher MIC breakpoint values for susceptibility because they achieve higher serum and site concentrations than other antibiotics. Because of this, a simple number comparison of the MIC between antibiotics should not be performed. The choice of antibiotic should be based on the knowledge of the MICs that are acceptable for a particular antibiotic–bacteria combination, the site of infection, the penetration of the antibiotic to the site of infection, as well as the clinical and economic parameters listed above. In the sample report in Figure 18-6, oxacillin (nafcillin) or cefazolin would be an acceptable choice for the treatment of *S. aureus* bacteremia in a patient without drug allergies because these agents are active against the infecting organism, have been demonstrated to be effective in the treatment of systemic staphylococcal infections, are relatively narrow-spectrum antibiotics, and are inexpensive. **Minicase 2** is an example illustrating the use of a bacterial culture and susceptibility report in the antibiotic decision-making process.

The decision regarding the antibiotics that will be reported on an individual susceptibility report for a bacterial isolate is typically based on input from a multidisciplinary committee (e.g., Antimicrobial Subcommittee, Infectious Diseases Subcommittee, Antimicrobial Stewardship Team) comprised of infectious diseases physicians, infectious diseases pharmacists, and representatives from the Infection Control Committee and the microbiology laboratory of a given hospital or institution. The decisions on which antibiotics to report on individual isolate susceptibility reports are often based on the hospital formulary, the level of control of antibiotic use that is desired, and the tests that are utilized by the microbiology laboratory for susceptibility testing. Tables that outline the antibiotics that should be routinely tested and reported for certain organisms can be found in the CLSI Performance Standards and Guidelines for Antimicrobial Susceptibility Testing.[21,26-28]

The methods utilized for reporting antibiotic susceptibility of bacteria for individual isolates include general reporting, selective reporting, and cascade reporting. *General reporting* involves reporting all antibiotics that were tested for susceptibility against the organism without any restrictions or analysis. *Selective reporting* involves the release of susceptibility information for a limited number of antibiotics, such as for those

MINICASE 2

Using Laboratory Test Results to Guide Choice of a Directed Antibiotic Regimen for the Treatment of Pyelonephritis

Diana J., a 27-year-old female, presents to the urgent care clinic with complaints of urinary frequency and urgency, pain on urination, and hematuria for the past two days. She also has had a fever of 101.6 °F and intractable nausea and vomiting during the past 24 hours. At presentation, she is febrile (102.3 °F), hypotensive (90/60), and lethargic; physical exam reveals right costovertebral angle and suprapubic tenderness. A urine dipstick performed in the clinic is leukocyte esterase positive, and a urine pregnancy test is negative. Because she is so ill-appearing, the clinic physician sends the patient to the local emergency department (ED) or admission. Her past medical history is significant for recurrent UTIs, with three episodes over the past six months that have required antibiotic therapy including trimethoprim–sulfamethoxazole and ciprofloxacin. She reports no known drug allergies. Upon admission to the ED, a urinalysis, urine culture, and blood cultures are performed.

QUESTION: What is an appropriate recommendation for antibiotic therapy for this patient?

DISCUSSION: This patient is presenting with pyelonephritis, an upper tract UTI, making the acquisition of a urinalysis, urine culture, and blood culture imperative in guiding antimicrobial treatment, especially because she has received multiple recent courses of antibiotics for her past UTIs, which put her at risk for infection with a resistant bacteria. Based on her presenting symptoms and the findings on her physical examination, she most likely has acute pyelonephritis. Because she is hypotensive on admission and is experiencing significant nausea and vomiting, she should initially be treated empirically with a parenteral antibiotic (e.g., ceftriaxone because she does not have any antibiotic allergies) with activity against the likely causative organisms of pyelonephritis and proven efficacy in the treatment of complicated UTIs. The patient can be continued on ceftriaxone or deescalated to parenteral cefazolin once the results of the urine culture and susceptibility tests are available.

Urinalysis: Yellow, cloudy; pH 7, specific gravity 1.015, protein negative, RBC trace, WBC 50–100/hpf, leukocyte esterase positive, nitrite positive

Midstream urine culture/susceptibility: >100,000 cfu/mL of *E. coli*

ANTIBIOTIC TESTED	MIC RESULT	CLSI INTERPRETATION
Ampicillin	>32 mcg/mL	R
Ampicillin–sulbactam	8 mcg/mL	S
Cefazolin	1 mcg/mL	S
Ceftriaxone	1 mcg/mL	S
Imipenem	1 mcg/mL	S
Gentamicin	0.5 mcg/mL	S
Ciprofloxacin	4 mcg/mL	R
Trimethoprim–sulfamethoxazole	>80 mcg/mL	R

R = resistant; S = susceptible.

Blood culture/susceptibility: *E. coli*

ANTIBIOTIC TESTED	MIC RESULT	CLSI INTERPRETATION
Ampicillin	>32 mcg/mL	R
Ampicillin–sulbactam	8 mcg/mL	S
Cefazolin	1 mcg/mL	S
Ceftriaxone	1 mcg/mL	S
Imipenem	1 mcg/mL	S
Gentamicin	0.5 mcg/mL	S
Ciprofloxacin	4 mcg/mL	R
Trimethoprim–sulfamethoxazole	>80 mcg/mL	R

R = resistant; S = susceptible.

available for routine use on the hospital formulary or for those useful for the treatment of a particular organism or infection type. An example of selective reporting would be the exclusion of cefazolin from the susceptibility report of a CSF sample growing *E. coli* because cefazolin is not a suitable treatment option for meningitis. *Cascade reporting* involves the release of susceptibility information for antibiotics that the hospital/committee considers to be first-line choices for the treatment of a particular organism or infection (usually narrow-spectrum and inexpensive), with the reporting of the susceptibility of second-line antibiotics (usually broad-spectrum and costly) only if the first-line agents are inappropriate for the treatment of the particular infection, or if the first-line agents are inactive against the infecting organism. This process may be utilized as an antimicrobial stewardship method to control the inappropriate use of broad-spectrum or expensive antibiotics.[48] An example of cascade reporting is the reporting of the susceptibility result of amikacin against *P. aeruginosa* only if the organism displays resistance to gentamicin and tobramycin, which are less expensive aminoglycoside agents.

Hospital Susceptibility Reports (Hospital Cumulative Antibiograms)

Most hospitals prepare and publish an annual cumulative report of antimicrobial susceptibility profiles of the bacteria that have been isolated from the patients within their hospital, healthcare system, or institution, called a *cumulative antibiogram*. The cumulative antibiogram usually reflects the antibiotic susceptibility patterns of isolates obtained from patients in the hospital who were admitted with an infection or developed an infection in the hospital (nosocomially acquired); although, some hospitals with a large outpatient population may also include isolates from patients in the surrounding community, usually in a separate outpatient table. The cumulative antibiogram is a useful tool for selecting *empiric* antibiotic therapy, where an antibiotic is selected based on the local susceptibility patterns of the most likely infecting organism causing the patient's infection (Table 18-7) while waiting for the results of culture and susceptibility tests, as described in **Minicase 3**.[48] Antibiotic therapy must often be initiated at the suspicion of infection because many infectious diseases are often acute where a delay in treatment may result in significant morbidity or mortality (e.g., meningitis and pneumonia). Once the culture and susceptibility results of the infecting bacteria are known, antibiotic therapy should be deescalated or directed, if necessary, to an agent with more targeted activity against the organism. For the cumulative antibiogram to be clinically useful, the susceptibility data from patient isolates should be appropriately collected, analyzed, and reported according to the CLSI guidelines, which are outlined in **Table 18-10**.[48]

The cumulative antibiogram contains information on the percent of isolated organisms that were susceptible to antibiotics tested over the time frame of the antibiogram, as illustrated in **Figure 18-7**.[48] This percent susceptibility information is derived by dividing the number of organisms susceptible to a particular antibiotic by the total number of single-patient isolates collected and reported (with duplicate patient isolates removed). The calculations can be performed either manually or using automated systems that have been programmed using appropriate definitions to remove duplicate patient isolates. The

MINICASE 3

Using the Cumulative Antibiogram to Guide Choice of an Empiric Antibiotic Regimen for Bacteremia

David M. is a 45-year-old male who sustained multiple traumatic injuries after a motorcycle accident. He has required multiple surgeries over the past 10 days for fracture stabilization. In the last 12 hours, he has spiked a temperature to 39 °C and has developed shaking chills. His other vital signs are stable, and his physical exam does not demonstrate any significant focal findings. Urinalysis, urine culture, and blood cultures are performed to determine the potential etiology for his new fever. In addition, a chest x-ray is performed, which does not demonstrate any pulmonary infiltrates. The laboratory calls the surgical floor later that day to report that the blood cultures are positive for gram-negative rods. The patient is allergic to penicillin (non-urticarial rash), and the hospital antibiogram is pictured in Figure 18-7.

QUESTION: What empiric antibiotic regimen should be used to treat this patient's gram-negative rod bacteremia?

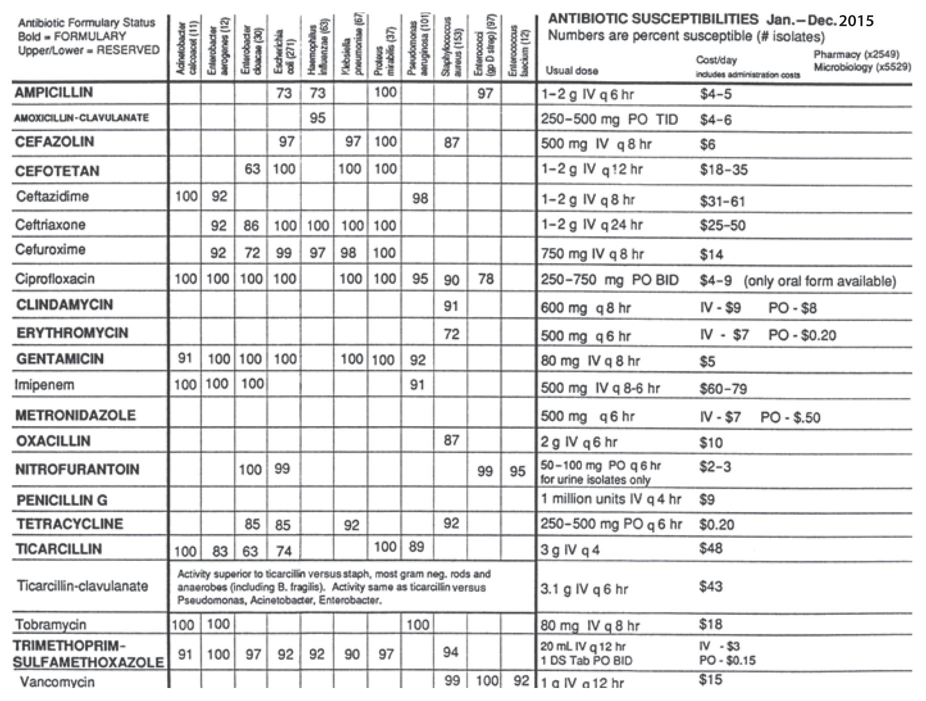
DISCUSSION: Nosocomial gram-negative bacteremia is a potentially life-threatening infection, which requires aggressive antibiotic therapy for treatment. The choice of whether to use monotherapy or combination therapy while waiting for culture and susceptibility results in this setting will often depend on the clinical condition of the patient and local susceptibility patterns. Combination antibiotic therapy might provide some antibacterial synergy, as well as provide coverage against a wide range of potential infecting bacteria. Based on the hospital antibiogram in Figure 18-7, it is desirable to choose antibiotics that demonstrate good activity (>85% susceptible) against gram-negative bacteria isolated at the institution (e.g., *P. aeruginosa*, *E. coli*, *K. pneumoniae*, *S. marcescens*, and *Enterobacter cloacae*) as well as choose agents that have demonstrated efficacy in the treatment of bacteremia. Because the patient is clinically stable and displays only a rash to penicillin therapy, some useful therapeutic options include meropenem, ceftazidime, cefepime, or ciprofloxacin monotherapy. If the patient begins to clinically deteriorate, an aminoglycoside, such as tobramycin, or a fluoroquinolone may be added to the carbapenem or cephalosporin while waiting for the culture and susceptibility results. The antibiotic regimen can be modified to more directed therapy, if possible, once the final culture and susceptibility results are available.

TABLE 18-10. CLSI Recommendations for Cumulative Antibiogram Development[48]

1. To serve as a continuously useful tool to guide appropriate empiric antibiotic therapy, the cumulative antibiogram should be compiled, analyzed, and reported at least annually.
2. Only the first clinical isolate of a given species of bacteria per patient per analysis period (yearly if that is the time frame of the antibiogram) should be included in the cumulative susceptibility report regardless of site of isolation, susceptibility pattern of the bacteria, or other phenotypic characteristics. The inclusion of duplicate clinical isolates from the same patient will lead to incorrect reporting of actual bacterial resistance patterns.
3. To provide a reasonable statistical estimate of susceptibility, only species of bacteria where at least 30 isolates have been collected, tested, and reported during the time period of the antibiogram should be included.
4. Data from isolates recovered during surveillance cultures (e.g., MRSA and VRE), environmental cultures, or other nonpatient sources should not be included in the antibiogram.
5. The cumulative susceptibility report should include all antibiotics that were tested for susceptibility, regardless if they were reported in the final susceptibility report for the individual patient.
6. Only bacterial isolates where all routine antibiotics have been tested for susceptibility should be included. Results of agents selectively or supplementally tested should not be included in the cumulative susceptibility report. For example, if only isolates resistant to primary agents were then analyzed for susceptibility to secondary agents, this will bias the resistance results toward higher levels of resistance to the secondary agents.
7. Data may be stratified and reported by age, unit in which the isolate was collected (e.g., MICU, SICU, outpatient clinic), or by anatomic site of collection (e.g., blood isolates, CSF isolates, and urine isolates) as long as duplicate patient isolates are removed and there are a sufficient number of isolates collected (>30) during the time frame of the antibiogram.

CSF = cerebrospinal fluid; MICU = medical intensive care unit; MRSA = methicillin-resistant *Staphylococcus aureus*; SICU = surgical intensive care unit; VRE = vancomycin-resistant enterococci.

data published in the cumulative antibiogram should be based on input from infectious diseases physicians, infectious disease pharmacists, the Infection Control Committee, the Pharmacy and Therapeutics Committee, and the microbiology laboratory of a given hospital or healthcare system. Cumulative antibiograms may contain separate data tables for reporting the susceptibility of gram-positive, gram-negative, and anaerobic bacteria; as well as separate data tables reporting the antibiotic susceptibility patterns of organisms isolated from patients in key patient care units (e.g., Burn Unit, Medical ICU, Pediatric Unit, Med-Surg Unit, outpatient clinic, nursing home), with particular infection types (e.g., susceptibility of bloodstream isolates or urinary tract isolates), with specific medical conditions (e.g., cystic fibrosis, transplant patients), or by organism (e.g., susceptibility of *S. aureus*). For some organisms, the cumulative antibiogram only will contain information regarding the presence of bacterial resistance mechanisms, particularly when routine susceptibility testing is difficult to perform, such as in the case of *H. influenzae* where the percentage of isolates that produce β-lactamase enzyme during the time



ANTIBIOTIC SUSCEPTIBILITIES Jan.–Dec. 2015
Numbers are percent susceptible (# isolates)
Pharmacy (x2549)
Microbiology (x5529)

Antibiotic Formulary Status Bold = FORMULARY Upper/Lower = RESERVED	Acinetobacter calcoaceticus (11)	Enterobacter aerogenes (12)	Enterobacter cloacae (30)	Escherichia coli (271)	Haemophilus influenzae (63)	Klebsiella pneumoniae (67	Proteus mirabilis (37)	Pseudomonas aeruginosa (101)	Staphylococcus aureus (153)	Enterococci (gp D strep) (97)	Enterococcus faecium (12)	Usual dose	Cost/day includes administration costs
AMPICILLIN				73	73		100			97		1–2 g IV q 6 hr	$4–5
AMOXICILLIN-CLAVULANATE					95							250–500 mg PO TID	$4–6
CEFAZOLIN				97		97	100		87			500 mg IV q 8 hr	$6
CEFOTETAN			63	100		100	100					1–2 g IV q 12 hr	$18–35
Ceftazidime	100	92						98				1–2 g IV q 8 hr	$31–61
Ceftriaxone		92	86	100	100	100	100					1–2 g IV q 24 hr	$25–50
Cefuroxime		92	72	99	97	98	100					750 mg IV q 8 hr	$14
Ciprofloxacin	100	100	100	100		100	100	95	90	78		250–750 mg PO BID	$4–9 (only oral form available)
CLINDAMYCIN									91			600 mg q 8 hr	IV - $9 PO - $8
ERYTHROMYCIN									72			500 mg q 6 hr	IV - $7 PO - $0.20
GENTAMICIN	91	100	100	100		100	100	92				80 mg IV q 8 hr	$5
Imipenem	100	100	100					91				500 mg IV q 8-6 hr	$60–79
METRONIDAZOLE												500 mg q 6 hr	IV - $7 PO - $.50
OXACILLIN									87			2 g IV q 6 hr	$10
NITROFURANTOIN			100	99						99	95	50–100 mg PO q 6 hr for urine isolates only	$2–3
PENICILLIN G												1 million units IV q 4 hr	$9
TETRACYCLINE			85	85		92			92			250–500 mg PO q 6 hr	$0.20
TICARCILLIN	100	83	63	74			100	89				3 g IV q 4	$48
Ticarcillin-clavulanate	Activity superior to ticarcillin versus staph, most gram neg. rods and anaerobes (including B. fragilis). Activity same as ticarcillin versus Pseudomonas, Acinetobacter, Enterobacter.											3.1 g IV q 6 hr	$43
Tobramycin	100	100						100				80 mg IV q 8 hr	$18
TRIMETHOPRIM-SULFAMETHOXAZOLE	91	100	97	92	92	90	97		94			20 mL IV q 12 hr 1 DS Tab PO BID	IV - $3 PO - $0.15
Vancomycin									99	100	92	1 g IV q 12 hr	$15

FIGURE 18-7. Example of a hospital antibiogram.

period of the cumulative antibiogram will be reported. Other information that may be incorporated into a cumulative antibiogram includes antibiotic dosing guidelines, recommended empiric antibiotic choices based on infection type, antibiotic cost data, etc.[48]

Surveillance Susceptibility Testing of Large Numbers of Isolates

Surveillance susceptibility testing is a useful method to monitor the susceptibility of bacteria to antimicrobial agents over time and can be performed in an individual hospital or within a geographic location (e.g., regionally, nationally, and internationally).[20] Surveillance studies typically report the overall susceptibility of the bacteria to particular antibiotics using CLSI breakpoints, along with other susceptibility parameters such as the MIC_{50} and the MIC_{90}. To determine the MIC_{50} or MIC_{90}, the MIC values from the bacterial population studied are arranged in ascending order where the MIC_{50} is the MIC value representing 50% of the bacterial population (the MIC value of the isolate that represents 50% of the bacterial population studied) and the MIC_{90} is the MIC that represents 90% of the bacterial population (the MIC value of the isolate that represents 90% of the bacterial population studied). The MIC_{90} value is usually higher than the MIC_{50} value. This information is useful for detecting the emergence of subclinical antibiotic resistance where the MIC_{50} and MIC_{90} of a particular agent may be increasing over time but are still below the MIC susceptibility breakpoint.

Additional Considerations When Interpreting Susceptibility Results

The successful treatment of a patient's infection involves an understanding of the interactions among the patient, the infecting organism, and the antibiotic. It is important to note that antimicrobial susceptibility testing only measures one of these factors, namely, the activity of the antibiotic against the infecting organism in a laboratory setting. The current methodologies for antibiotic susceptibility testing are unable to reproduce the interaction between the antibiotic and the bacteria at the site of infection where a multitude of host factors (e.g., immune system function, concomitant disease states, etc.) and drug factors (e.g., pharmacokinetic parameters including concentration of free drug at the site of infection and protein binding) play an integral role.

FUNGI

Fungi are classified as one of the six kingdoms of life. There are approximately 500 named species of fungi that are known to cause infection in humans and other vertebrate animals.[49] Approximately 50 fungal species are associated with infections in healthy subjects and the majority of fungal infections occur in immunocompromised or debilitated patients by organisms that are part of the normal human flora. However, an increasing number of serious and life-threatening opportunistic infections are being caused by ubiquitous environmental molds.

One of the most challenging and frustrating aspects of diagnostic medical mycology is the terminology, taxonomy, classification, and nomenclature of fungi.[49] For example, the correct name for a species of fungi is that which was published earliest and met the requirements in the International Code of Botanical Nomenclature (http://www.iapt-taxon.org). All subsequent names are considered synonyms; however, exceptions do exist, particularly when a later name is more commonly used than the earlier name or if research requires a species to be transferred to a different genus. Changes have occurred between kingdoms as well. For example, members of the genus *Pneumocystis*, which were originally placed in the kingdom Protozoa, are now reclassified as fungi and placed in the phylum Ascomycota. The common human pathogen from this genus is *P. jirovecii*. Because of these issues, the reader is referred to the latest editions of standard microbiology textbooks and reference manuals (e.g., *Manual of Clinical Microbiology*, American Society for Microbiology Press) for more detailed information on taxonomy and classifications of fungi. In addition, a glossary of common mycological terms is often included.[49]

Fungi are eukaryotic and can be either unicellular or multicellular organisms. Fungi have cell walls composed mainly of chitins, glucan, and mannam with a membrane-bound cell nucleus with chromosomes. The dominant sterol in the cytoplasmic membrane of fungi is ergosterol, compared to cholesterol in mammalian cells. These organisms are heterotropic (e.g., require exogenous energy sources) and can reproduce by either asexual (involving mitosis) or sexual (involving meiosis) cell division. Fungi may exist in a morphologic form that results from sexual reproduction (teleomorph, or perfect state) and a form that results from asexual reproduction (anamorph or imperfect state), where each of the forms has its own name (e.g., the sexual form of *Scedosporium apiospermum* complex is *Pseudallescheria boydii*). The use of separate anamorph and teleomorph species names ended in January 2013, and all legitimate names for the species can be used. A formalization of the preferred species name will be formalized by 2016.[49]

Fungi have traditionally been categorized into mold, yeast, or dimorphic fungi based on morphological and structural features (**Table 18-11**).[49-53] *Molds* (or moulds) are long, cylindrical, and threadlike (filamentous) fungi that form multicellular mycelium or thallus, an intertwined mass of branching hyphae (tube-like extensions or filament-like cells), with septa (having cross walls; being septate) or pauciseptate. An asexual spore (conidium) is produced on conidiophores, a specialized hyphal structure that serves as a stalk, and macroconidia and microconidia may be present. Thermally, monomorphic molds can be divided into four groups: (1) Zygomycetes; (2) dematiaceous fungi; (3) dermatophytes; and (4) hyaline hyphomycetes. Zygomycetes have broad hyphae that are almost nonseptate with asexual spores (sporangiospores) formed by cleavage in a saclike structure (sporangium). The most common Zygomycetes observed in the clinical laboratory are from the order Mucorales, which are associated with severe fungal infections referred to as *mucormycosis*. Two genera from the order

TABLE 18-11. Categorization of Selected Medically Important Fungi[53,a]

YEASTS AND YEAST-LIKE ORGANISMS		**DIMORPHIC FUNGI**[b]	
Candida spp.	*Saccharomyces cerevisiae*	*Histoplasma capsulatum*	*Sporothrix schenckii complex*[d]
Cryptococcus spp.	*Trichosporon* spp.	*Blastomyces dermatitidis*	*Talaromyces marneffei*
Rhodotorula spp.	*Blastoschizomyces capitatus*	*Paracoccidioides brasiliensis*	

MOLDS

Zygomycetes[c]	**Dematiaceous Fungi**	**Dermatophytes**	**Hyaline Hyphomycetes**
Rhizopus spp.	*Fonsecaea pedrosoi*	*Microsporum* spp.	*Coccidioides* spp.[b]
Mucor spp.	*Fonsecaea compacta*	*Trichophyton* spp.	*Hormographiella aspergillata*
Rhizomucor spp.	*Rhinocladiella* spp.	*Epidermophyton floccosum*	*Emmonsia* spp.
Lichtheimia corymbifera complex	*Phialophora verrucosa*		*Aspergillus* spp.
Apophysomyces elegans	*Pleurostomophora richardsiae*		*Penicillium* spp.
Saksenaea vasiformis	*Phaeoacremonium parasiticum*		*Paecilomyces* spp.
Cunninghamella bertholletiae	*Phialemonium* spp.		*Scopulariopsis* spp.
Basidiobolus spp.	*Cladophialophora* spp.		*Acremonium* spp.
Conidiobolus coronatus	*Pseudallescheria boydii* (sexual state)		*Fusarium* spp.
	Scedosporium apiospermum (asexual state)		*Lecythophora* spp.
	Scedosporium prolificans		
	Ochroconis gallopava		
	Exophiala jeanselmei		
	Complex		
	Exophiala dermatitidis		
	Hortaea werneckii		
	Stachybotrys chartarum		
	Curvularia spp.		
	Bipolaris spp.		
	Exserohilum spp.		
	Alternaria spp.		

[a] Refer to reference 53 for further details and other organisms not listed.

[b] *Coccidioides immitis* is often placed with dimorphic fungi (listed in this table under hyaline hyphomycetes because it does not produce yeast-like colonies or cells at 35–37 °C on routine mycology agar).

[c] Results from molecular studies have recommended that the phylum Zygomycota be divided among a new phylum, Glomeromycota and four subphyla, changing the class Zygomycetes to glomeromycetes. Many textbooks continue to use Zygomycetes until the taxonomy is definitively resolved. Among the subphylum Mucormycotina, the order Mucorales includes the genera Rhizopus, Mucor, Rhizomucor, and Lichtheimia (formerly Absidia). The subphylum Entomophthoromycotina contains the order Entomophthorales, which includes genera Basidiobolus and Conidiobolus.

[d] *Sporothrix schenckii* grows as a dematiaceous mold when incubated at 25–30 °C but is yeast-like at 35–37 °C. It is commonly categorized among dimorphic fungi but is often considered as a dematiaceous mold.

Entomophthorales, *Basidiobolus* and *Conidiobolus*, are less commonly observed Zygomycetes but are responsible for subcutaneous infections in otherwise healthy individuals. Dematiaceous fungi produce dark colored colonies of olive, brown, gray or black due to melanin pigment in the cell walls. Some of the common infections associated with dematiaceous fungi include chromoblastomycosis, phaeohyphomycosis, and mycetoma. Dematiaceous fungi also cause tinea nigra and black piedra. Dermatophytes are most often associated with superficial fungal infections (tinea or ringworm) of the skin, hair, and nails. These filamentous fungi colonize the outermost layer of the skin and digest keratin as a source of nutrients. The three genera (*Microsporum*, *Trichophyton*, and *Epidermophyton*) are differentiated by their conidium formation (macroconidia or microconidia). Hyaline hyphomycetes are colorless, septate hyphae molds that produce conidia that may be either colorless or pigmented. *Coccidioides immitis* and *Coccidioides posadasii* are known pathogens from this group while most other organisms cause opportunistic infections in immunocompromised patients.

Yeasts (and yeast-like organisms) appear as round or oval cells that are unicellular and generally reproduce at their surface by budding (blastoconidia). Some produce pseudohyphae (an elongated chain of cells, like a chains of sausages, resembling hyphae; however, borders between cells are delineated by marked constrictions), while others have true hyphae (tend to be straighter and without constrictions at the septa), which may be septate or without septa (aseptate). Ascospores, a sexual spore in a saclike structure (ascus), are produced by only some yeast. Yeasts are the most frequent encountered fungi in the clinical microbiology laboratory and are considered opportunistic pathogens. *Candida* spp. and *Cryptococcus* spp. are among the most common yeasts causing fungal infections. Yeasts are not considered as a formal taxonomic group but as a growth form of unrelated fungi (members of the phyla Basidiomycota and Ascomycota).

Dimorphic fungi have two distinct morphological forms where their growth forms can change from a multicellular mold (in their natural environment or when cultured at 25–30 °C) to budding, unicellular yeasts (during tissue invasion or when cultured at 35–37 °C). Medically important dimorphic fungi include *Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Paracoccidioides brasiliensis*, *S. schenckii*, and *Talaromyces marneffei* (formerly *Penicillium marneffei*). All of these fungi are considered pathogenic and must be handled with caution in the clinical laboratory.

The Identification of Fungi

The following section provides a brief summary of the common methods currently used in diagnostic testing of medically important fungi.[50-53] Fungal identification has traditionally been based on morphological characteristics such as the color of the colonies, the size and shape of cells, the presence of a capsule, and the production of hyphae, pseudohyphae, or chlamydospores. Culture remains the "gold standard" in most clinical microbiology laboratories and is the only method that allows subsequent susceptibility testing. Deoxyribonucleic acid (DNA) sequence analysis and molecular characteristics are being rapidly explored and gaining a larger routine role in fungal identification, particularly when morphology-based identification is atypical, confusing, or not helpful (e.g., organisms that fail to sporulate) and in cases where precise identification is required (e.g., epidemiological studies).[54,55] Because no one test is perfect, it is often necessary to perform several diagnostic tests (both morphological and genotypic methodologies) to maximize the accuracy of fungal identification. Laboratory diagnosis of fungal infections includes direct microscopic examination, isolation in culture, morphologic identification, biochemical, serologic, surrogate markers, and molecular diagnostic testing.[52-55] As with all types of infections, appropriate biological specimens need to be selected, collected, and transported to the laboratory for immediate processing.[50] Because different fungi are capable of causing infection at a number of anatomical sites, specimens from the site of infection as well as peripheral blood specimens should be considered and submitted for culture and microscopic examination. Communication with the laboratory regarding the clinical infection and suspected fungi is important and may be useful for determining how best to process specimens safely and efficiently, including pretreatment and staining procedures, selection and incubation of media, and choice of additional diagnostic testing. Early identification of the infecting fungal pathogen may have direct diagnostic, epidemiologic, prognostic, and therapeutic implications.

The first step is usually identifying yeast-like fungi (pasty, opaque colonies) from molds (large, filamentous, colonies that vary in texture, color, and topography). Drawings, color plates, and brief descriptions found in standard textbooks can serve as a guide and assist in the preliminary identification of fungi seen on direct microscopic examination of clinical specimens.[52,53] Microscopic examination of the clinical specimen can delineate morphologic features (**Table 18-12**) and often provides preliminary identification of many fungi (e.g., *Aspergillus* spp., Zygomycetes, dematiaceous molds). Microscopic morphology can often provide definitive identification of a mold whereas the addition of biochemical tests, serology, and NA–based molecular testing are usually needed for identifying the genus and species of most yeast and yeast-like fungi. Direct microscopic examination of properly stained clinical specimens and tissue sections is usually the most rapid (within a few minutes or hours) and cost-effective method for a preliminary diagnosis of fungal infection. In addition, microscopic detection of fungi can assist the laboratory in the selection of media and interpretation of culture results.

The Gram stain that is typically used for bacterial processing may also allow the detection of most fungi, especially *Candida* spp., because the size of the smallest fungi is similar in size to large bacteria; the presence of budding cells can also be observed. A wide range of stains are available (**Table 18-13**) to assist in the rapid detection of fungal elements.[51-53] A common approach to wet preparations of specimens or smeared dried material is to use a 10% solution of potassium hydroxide (KOH) with or without fluorescent calcofluor white (CFW) stain. The strong alkaline KOH solution digests tissue elements to allow better visualization of the fungi, while the CFW stain binds to chitin and polysaccharides in the fungal cell wall allowing it to appear white under ultraviolet light. Specific staining techniques are often used to outline morphologic features that are diagnostic and distinctive of the suspected fungal organism (e.g., India ink stain for detection of a polysaccharide capsule of *Cryptococcus neoformans*). In suspected cases of histoplasmosis, the Giemsa or Wright stain is useful for detecting intracellular yeast cells within macrophages from blood or bone marrow specimens.

Histopathologic stains are extremely valuable for identifying fungal elements in tissues and host tissue reactions to fungal infection.[55,56] Histology laboratories commonly use stains such as hematoxylin and eosin for these general purposes. Periodic acid-Schiff and Gridley fungus stains can also assist in visualization of fungal elements, especially if debris is present in the tissue background. Special stains such as Gomori methenamine silver, mucicarmine, and Fontana-Masson are

TABLE 18-12. Laboratory Testing and Characteristic Features Used in the Diagnosis of Selected Opportunistic and Pathogenic Fungi[52,53,55]

		MICROSCOPIC MORPHOLOGIC	MORPHOLOGIC FEATURES IN CULTURE		SEROLOGIC TESTS		
FUNGAL ORGANISM	**POTENTIAL CLINICAL SPECIMENS**	**FEATURES OF CLINICAL SPECIMENS**	**MACROSCOPIC**	**MICROSCOPIC**	**ANTIGEN**	**ANTIBODY**	**ADDITIONAL TESTS FOR IDENTIFICATION**
Candida spp.	Blood; bone marrow; catheter sites; eye; respiratory sites; skin, nails, mucous membrane; urine; vaginal, urethral, prostatic secretions or discharge; multiple systemic sites	Round to oval budding yeasts (3–6 µm in diameter), singly, in chains, or in small loose clusters; true hyphae (no or slight constrictions at the septa) and pseudohyphae (5–10 µm in diameter; chains of elongated blastoconidia) when invading tissues; blastoconidia develop along the sides of either type of hyphae *Candida glabrata* slightly smaller (2–5 µm in diameter) than other species and does not produce any hyphal forms	Variable morphology; colonies usually pasty, white to tan and opaque; may have smooth or wrinkled morphology	Clusters of blastoconidia, pseudohyphae and terminal chlamydospores in some species	Part of (1,3)-β-D-glucan panfungal detection	EIA test for detection of *Candida* mannan antigen and anti-mannan antibody; both the antigen and antibody test be performed together to maximize early diagnosis	Germ tube production by *Candida albicans*, *Candida dubliniensis*, and *Candida stellatoidea* PNA-FISH MALDI-TOF MS Gene sequencing Carbohydrate assimilation Morphology on corn meal agar, CHROMagar, rapid trehalose test
Cryptococcus neoformans	Most commonly in CSF and blood; bone marrow; catheter sites; respiratory sites; skin, mucous membrane; urine; multiple systemic sites	Spherical (football shaped) or round, budding yeasts of variable size (2–15 µm) with thin dark walls; thick capsule may or may not be present; no hyphae or pseudohyphae	Colonies are shiny, mucoid, dome-shaped, and cream to tan in color	Budding spherical cells of varying size; capsule present; no pseudohyphae; cells may have multiple narrow-based buds	Latex antigen or EIA test for polysaccharide antigen		India ink can demonstrate capsule in ~50% of cases on smeared specimens Tests for urease (+), phenoloxidase (+), and nitrate reductase (-) Mucicarmine and melanin stains in tissue
Aspergillus spp.	CSF; eye; respiratory sites; skin, mucous membrane; urine; multiple systemic sites	Septate with uniform diameter (3–6 µm); dichotomously branched hyphae at 45° angles; tend to grow in radial fashion (like spokes on a wheel)	Varies with species; *Aspergillus fumigates*: blue-green to gray; *Aspergillus flavus*: yellow to green; *Aspergillus niger*: black with white margins and yellow surface mycelium	Varies with species; conidiophores with enlarged vesicles covered with flask-shaped metulae or phialides; hyphae are hyaline and septate	Galactomannan detection is useful tool in the diagnosis of invasive aspergillosis Part of (1,3)-β-D-glucan panfungal detection	Useful for chronic and allergic aspergillosis	Gene sequencing Molecular techniques are attractive as alternative methods but are mainly in-house systems without standardization being available

(continued)

TABLE 18-12. Laboratory Testing and Characteristic Features Used in the Diagnosis of Selected Opportunistic and Pathogenic Fungi[52,53,55], cont'd

		MICROSCOPIC MORPHOLOGIC	MORPHOLOGIC FEATURES IN CULTURE		SEROLOGIC TESTS		
FUNGAL ORGANISM	**POTENTIAL CLINICAL SPECIMENS**	**FEATURES OF CLINICAL SPECIMENS**	**MACROSCOPIC**	**MICROSCOPIC**	**ANTIGEN**	**ANTIBODY**	**ADDITIONAL TESTS FOR IDENTIFICATION**
Zygomycetes	CSF; eye; respiratory sites; skin, mucous membrane; multiple systemic sites	Broad, thin-walled, pauciseptate hyphae (6–25 µm) with nonparallel sides and branching irregularly, nondichotomous, and at various angles; hyphae stain poorly with GMS stain and often stain well with H&E stain	Colonies are rapid growing, wooly or fluffy colonies (cotton candy-like), and gray-black in color	Differentiation of various genera based on presence and location (or absence) of rhizoids, nature of sporangiophores, shape of columella, appearance of an apophysis, and size and shape of the sporangia			
Dematiaceous molds	CSF; eye; respiratory sites; skin, mucous membrane; multiple systemic sites	Pigmented (brown, tan, or black) polymorphous hyphae (2–6 µm in diameter) with single septa and in chains of swollen rounded cells	Colonies are usually rapidly growing, wooly, and gray, olive, black, or brown in color	Varies depending on genus and species; hyphae are pigmented; conidia may be single or in chains, smooth or rough, and dematiaceous			
Histoplasma capsulatum	Blood; bone marrow; CSF; eye; respiratory sites; skin, mucous membrane; urine; multiple systemic sites	Small (2–4 µm in diameter), oval to round budding cells; often clustered within histiocytes or intracellular (macrophages, monocytes) Can be difficult to differentiate Histoplasma from other small yeasts (e.g., *Candida* spp.) or some parasites	Colonies are slow growing and white or buff brown in color (25 °C); yeast phase colonies (37 °C) are smooth, white, and pasty	Thin, septate hyphae that produce tuberculate macroconidia and smooth wall microconidia (25 °C); small, oval, budding yeasts produced at 37 °C	Various methods for initial antigen screening	Antigen screening test should be validated with antibody testing	AccuProbe may be useful for confirming identification Demonstration of temperature-regulated dimorphism Exoantigen and nucleic acid probe tests allow identification without phase conversion

(*continued*)

TABLE 18-12. Laboratory Testing and Characteristic Features Used in the Diagnosis of Selected Opportunistic and Pathogenic Fungi[52,53,55], cont'd

		MICROSCOPIC MORPHOLOGIC	MORPHOLOGIC FEATURES IN CULTURE		SEROLOGIC TESTS		
FUNGAL ORGANISM	**POTENTIAL CLINICAL SPECIMENS**	**FEATURES OF CLINICAL SPECIMENS**	**MACROSCOPIC**	**MICROSCOPIC**	**ANTIGEN**	**ANTIBODY**	**ADDITIONAL TESTS FOR IDENTIFICATION**
Blastomyces dermatitidis	CSF; respiratory sites; skin, mucous membrane; urine; multiple systemic sites	Large (8–15 µm in diameter) and spherical, thick-walled (commonly referred to as "double contoured") cell; each yeast cell produces a single bud, attached to parent cell on a broad base	At 25–30 °C, colonies vary from cottony or fluffy white with aerial mycelium (first 2–3 days) then to a glabrous, tan, nonconidiating colony When grown at 35–37 °C, cream to tan color, wrinkled, folded, and glabrous (waxy in appearance)	On a wet mount at 25–30 °C, septate hyphae with one-celled smooth conidia (lollipop-like appearance); at 37 °C, large, thick walled and double contoured yeast-like cell, budded on a broad base	Sandwich EIA available to detect antigenuria and antigenemia in disseminated blastomycosis	Immunodiffusion test is the most useful method	AccuProbe may be useful for confirming identification Demonstration of temperature-regulated dimorphism Exoantigen and nucleic acid probe tests allow identification without phase conversion
Coccidioides immitis/ posadasii	Blood; bone marrow; CSF; eye; respiratory sites; skin, mucous membrane; urine; multiple systemic sites	Round, thick-walled spherules that vary in size (20–200 µm in length); mature spherules contain small (2–15 µm in diameter) endospores; septate hyphae, barrel-shaped arthroconidia may be seen in cavitary and necrotic lesions	Great variation in morphology; at 25 °C or 37 °C, colonies initially appear moist and glabrous, rapidly develops a white, cottony aerial mycelium, which becomes gray-white to a tan or brownish	Hyaline hyphae with rectangular (barrel-shaped) arthroconidia separated by empty disjunctor cells	EIA using antibodies against *Coccidioides galactomannan*	Various methods (ID, complement-fixation most reliable) for initial antibody screening (principle antigen used in these test is coccidioidin)	AccuProbe may be useful for confirmation of unknown isolates as *Coccidioides* species (but does not distinguish between two species of *Coccidioides*) Exoantigen and nucleic acid probe tests allow identification without phase conversion
Sporothrix schenckii	Blood; CSF; respiratory sites; skin, mucous membrane; multiple systemic sites	Small (2–6 µm in diameter) yeast-like cells of varying sizes and shapes (oval or round or cigar shaped); single or multiple elongated "pipe stem;" bud is on a narrow base	At 25–30 °C, colonies are initially small, smooth, moist and white to pale orange to orange-gray with no cottony aerial hyphae; later, colonies become moist, wrinkled, leathery, or velvety and darken to brown or black; at 35–37 °C, colonies are white to tan, dry, smooth and yeast-like	At 25–30 °C, thin or narrow, septate, and branching, with slender, tapering conidiophores rising at right angles; conidia borne in rosette-shaped clusters at the end of the conidiophores; at 35–37 °C, variable-sized round, oval, and fusiform budding yeasts (cigar bodies)		LA test is commercially available (particularly for disseminated cases)	Demonstration of temperature-regulated dimorphism Exoantigen and nucleic acid probe tests allow identification without phase conversion

(continued)

TABLE 18-12. Laboratory Testing and Characteristic Features Used in the Diagnosis of Selected Opportunistic and Pathogenic Fungi[52,53,55], cont'd

		MICROSCOPIC MORPHOLOGIC	MORPHOLOGIC FEATURES IN CULTURE		SEROLOGIC TESTS		
FUNGAL ORGANISM	**POTENTIAL CLINICAL SPECIMENS**	**FEATURES OF CLINICAL SPECIMENS**	**MACROSCOPIC**	**MICROSCOPIC**	**ANTIGEN**	**ANTIBODY**	**ADDITIONAL TESTS FOR IDENTIFICATION**
Talaromyces marneffei	Blood; bone marrow; CSF; respiratory sites; skin, mucous membrane; urine; multiple systemic sites	Oval (2.5–5 µm in length) or elongated or cylindrical, curved yeast-like cells; found within histiocytes (intracellular); has visible septa and budding does not occur (reproduces by fission [arthroconidium-like])	Colonies are flat, powdery to velvet, and tan, and then produce diffusible red–yellow pigment at 25–30 °C; at 35–37 °C, colony is soft, white to tan, dry, yeast-like	At 25–30 °C, smooth conidiophores with four to five terminal metulae bearing phialides; chains of short, narrow extensions connect the round to oval conidia in a "paint brush" distribution; at 35–37 °C, arthroconidial yeast cells divide by fission and may elongate			Demonstration of temperature-regulated dimorphism
Pneumocystis jirovecii	Respiratory sites; multiple systemic sites	Cysts are round, ovoid, or collapsed crescent shaped (4–7 µm in diameter); stains should be used to visualize cyst forms for diagnosis; trophozoites are small (1–4 µm in diameter), pleomorphic forms	Not applicable	Not applicable		Useful for epidemiology studies but not for diagnosis	Immunofluorescent stain, Gomori methenamine silver, Giemsa, toluidine blue stains

CSF = cerebrospinal fluid; EIA = enzyme immunoassay; ID = immunodiffusion; LA = latex agglutination.

TABLE 18-13. Stains Used to Enhance the Direct Microscopic Detection of Fungi[51-53]

STAIN (ABBREVIATION)	DETECTION	CHARACTERISTICS/COMMENTS
Alcian blue	*Cryptococcus neoformans* (in CSF)	Histopathologic stain for mucin
Brown and Brenn (B&B)	*Nocardia, Actinomadura,* etc. (demonstrates the bacterial filaments of the actinomycetes)	Stains fungi blue
Calcofluor white (CFW)	Most fungi including *Pneumocystis jirovecii* (cysts)	Binds to chitin in fungal cell wall and fluoresces bluish white against dark background; requires fluorescent microscope; mixed with KOH for easier and rapid observation of fungi
Fontana-Masson (FM)	Dematiaceous fungi, *Cryptococcus neoformans,* and *Cryptococcus gattii*; may also be useful for *Aspergillus fumigates, Aspergillus flavus, Trichosporon* spp., and some Zygomycetes	Stains fungi brown to black against reddish background; demonstration of melanin or melanin-like substances in the lightly pigmented agents of phaeohyphomycosis
Giemsa or Wright	Visualization of intracellular *Histoplasma capsulatum*; trophic forms of *Pneumocystis jirovecii*; fission yeast cells of *Talaromyces marneffei*	Stains blue-purple (fungi and bacteria); examination of bone marrow or peripheral blood smears for disseminated disease
Gomori methenamine silver (GMS)	Most fungi in histopathologic sections; *Pneumocystis jirovecii* (respiratory specimens)	Detects fungi elements; however, requires specialized staining method; stains hyphae and yeast forms gray to black against a pale green or yellow background
Gram	Yeast and pseudohyphae appear gram-positive and hyphae (septate and aseptate) appear gram-negative	Commonly performed on clinical specimens; some fungi stain poorly (e.g., *Cryptococcus* spp., *Nocardia*)
Gridley fungus (GF)	Most fungi in histopathologic sections	Fungi stain purplish red; filaments of Actinomycetes are not stained
Hematoxylin and eosin (H&E)	General purpose histopathologic stain; best method for visualizing host tissue reactions to infecting fungus	Stains some fungal elements violet to bluish purple in contrast to lighter background; *Aspergillus* spp. and Zygomycetes stain well; some fungi difficult to differentiate from background
Immunohistochemical	*Aspergillus* spp., *Candida albicans, Pneumocystis jirovecii*	Commercial antibodies used in the immunohistochemical diagnosis of fungal infections, especially to distinguish fungal elements on atypical appearing tissue sections
India ink, nigrosin	*Cryptococcus neoformans* (in CSF)	Sensitivity is <50% of meningitis cases
Modified acid-fast	*Nocardia* (filaments are partially acid-fast and stain pink) and some isolates of *Blastomyces dermatitidis*	Actinomyces and other actinomycetes are negative
Mucicarmine	*Cryptococcus neoformans* (capsular material); cell walls of *Blastomyces dermatitidis* and *Rhinosporidium seeberi*	Histopathologic stain for mucin; capsular material stains deep rose to red; tissue elements stain yellow
Periodic acid–Schiff (PAS)	Histopathologic stain for fungi, especially yeast cells and hyphae in tissue; commonly used stain by dermatopathologists	Fungal elements stain bright pink–magenta or purple against orange background (picric acid counterstain) or green background (if light green used); hyphae of molds and yeast can be readily distinguished; demonstrates double-contoured refractile wall of *Blastomyces dermatitidis*; *Nocardia* do not stain well
Potassium hydroxide (KOH)	Most fungi (more readily visible)	Used to dissolve tissue material allowing more visible fungal elements
Toluidine blue	*Pneumocystis jirovecii* (respiratory specimens: biopsy or BAL)	Stains cysts of *Pneumocystis jirovecii* reddish blue or dark purple against light blue background

BAL = bronchoalveolar lavage; CSF = cerebrospinal fluid.

useful for enhancing the detection of specific fungal elements (Table 18-13).[51-53]

Culture remains the gold standard for isolation and identification of fungi suspected of causing infection. Petri plates are preferred over screw-cap tubes because of the larger surface area and dilution of inhibitory substances in the specimens. However, for laboratory safety reasons, most thermally dimorphic fungi (e.g., *Histoplasma*, *Blastomyces*, *Paracoccidioides*, *T. marneffei*) and *Coccidioides* spp. are pathogenic and should be grown on slants (i.e., avoid the use of Petri plates and slide culture). A variety of media are available for the isolation and cultivation of yeasts and molds (**Table 18-14**).[50,51,53] Sabouraud

TABLE 18-14. Examples of Various Media Used for the Recovery of Fungi from Clinical Specimens[50,51,53]

GROWTH MEDIUM	COMMENTS AND USES
Primary Media Without Antibacterials or Antifungals	
Brain heart infusion (BHI) agar	Enriched media used for cultivation and isolation of all fungi; designed to enhance the recovery of fastidious dimorphic fungi than does SDA
Littman Oxgall agar	General purpose selective medium for isolation of all fungi. Restricts the spreading of fungal colonies; contains crystal violet and streptomycin to inhibit bacteria growth
Sabouraud dextrose agar (SDA)	Supports primary growth or sporulation and provides classic pigment and morphology
SDA, Emmons modification	Compared to SDA, Emmons Modification contains 2% (versus 4%) glucose and has a pH of 6.9 (versus pH 5.6 [slightly acidic])
SADHI medium	Enriched media using combined ingredients of BHI and SDA; supports growth of all fungi; designed for better recovery of fastidious dimorphic fungi than does SDA
Primary Media with Antibacterials or Antifungals	
Any of the above media	Usually with chloramphenicol (inhibits gram-negative and gram-positive bacteria) with or without gentamicin (inhibits gram-negative bacteria); cycloheximide added to inhibit sensitive fast-growing saprophytic fungi
Inhibitory mold agar (IMA)	Enriched media providing better recovery of fastidious fungi than does SDA; usually contains chloramphenicol; some formulations contain gentamicin
Mycosel or mycobiotic	Selective medium containing chloramphenicol or cycloheximide primarily used for isolation of dermatophytes; can also be used for isolation of other pathogenic fungi from contaminant specimens
Selective/Differential Media	
Dermatophyte test medium (DTM) or dermatophyte identification medium (DIM)	Screening medium for the recovery, selection, and differentiation of dermatophytes (e.g., *Microsporum, Trichophyton, Epidermophyton*) from hair, skin, and nail (cutaneous) specimens; contains chloramphenicol, gentamicin, and cycloheximide; other saprophytic fungi and *Aspergillus* spp. can grow on this medium (thus, it is recommended only as a screening medium)
Yeast extract phosphate	Used for isolation and sporulation of slowly growing dimorphic fungi (i.e., *Histoplasma capsulatum* and *Blastomyces dermatitidis*) from contaminated specimens; contains chloramphenicol and ammonium hydroxide to suppress bacteria, molds, and yeasts and further permit detection of dimorphic fungi
CHROMagar candida[a]	Chromogenic media used for direct and rapid differentiation of many clinically important yeast spp; contains chloramphenicol to inhibit bacteria and is available with or without fluconazole (selection of fluconazole-resistant *Candida krusei*); CHROMagar differentiates more *Candida* spp. than CAN2; useful in identifying mixed cultures of yeasts
ChromID candida agar (CAN2)[a]	Chromogenic media used for direct and rapid identification of *Candida albicans* versus other species of yeasts; useful in identifying mixed cultures of yeasts
Specialized Media	
Cornmeal agar (CMA)	CMA with 1% dextrose used for the cultivation of fungi and differentiation of *Trichophyton mentagrophytes* from *Trichophyton rubrum* (based on pigment production); CMA with Tween 80 used for the cultivation and differentiation of *Candida* spp. (based on mycelial characteristics); Tween 80 promotes growth and production of red pigment by *Trichophyton rubrum*
Potato dextrose agar (PDA) or potato flake agar (PFA)	PDA is used to stimulate conidium production by fungi and enhance pigment production by some dermatophytes; PDA is most commonly used with slide culture technique to view morphological characteristics; PFA used for the simulation of conidia of fungi; PFA may include cycloheximide and chloramphenicol
Rapid sporulation agar (RSA)	Cultivation of ascosporogenous yeasts (e.g., *Saccharomyces cerevisiae*); contains chloramphenicol and chlortetracycline to inhibit bacteria and cycloheximide to inhibit saprobic fungi
Scedosporium-selective medium	May be necessary to detect *Scedosporium* in respiratory specimens of cystic fibrosis patients
Niger seed or bird seed and esculin base medium (EBM)	Selective and differential medium for isolation of *Cryptococcus* spp., especially *Cryptococcus neoformans* and *Cryptococcus gattii*

[a] Several commercial sources of yeast chromogenic agar media are available.[51] CHROMagar Candida and chromID Candida agar are examples of selected chromogenic agar products that are approved by the FDA for use in U.S. laboratories. *Candida* spp. identified with chromogenic agar differs by medium and manufacturer. Further testing is required for final identification.

dextrose and brain heart infusion or BHI agar are enriched media commonly recommended to permit the growth of yeasts and molds. Several media, with (selective) and without (nonselective) inhibitory agents, should be used because no one media is adequate for all the different types of specimens or organisms. Antibiotics such as chloramphenicol or gentamicin are included as inhibitory substances of most bacterial contaminants whereas cycloheximide is used to inhibit saprophytic fungi and prevent the overgrowth of contaminating molds. Nonselective media (without inhibitory agents) should be used with specimens from sterile sites and when suspected fungi are likely to be inhibited by cycloheximide (e.g., *Aspergillus* *fumigatus*, *Fusarium*, *Scopulariopsis*, *C. neoformans/gattii*, some *Candida* spp., most Zygomycetes) or by antibiotics (e.g., *Nocardia* or other filamentous bacteria). Direct microbiological examination (outlined above) of clinical specimens can assist in the selection of media based on specimen type and suspected pathogen. In addition, the choice of media will be influenced by the patient population, local endemic pathogens, cost, availability, and laboratory preferences.

Proper incubation temperature and sufficient incubation time are also needed to optimize the recovery of medically important fungi from clinical specimens. Inoculated media should be incubated aerobically at 30 °C. If an incubator at that temperature is not available, then 25 °C (room temperature) can be considered. Other temperatures (e.g., 35–37 °C for thermally dimorphic organisms) should be reserved for selected fungi that prefer a higher temperature. In general, yeasts are detected within five days or less, dermatophytes within one week, and dematiaceous and dimorphic fungi between two to four weeks. Cultures should be regularly reviewed (e.g., every day the first week, every two to three days the second week, twice during the third week, once weekly thereafter) to account for the growth rates and identification of fungi. Incubating cultures for four weeks is usually necessary before no growth of fungus should be considered. Several factors influence the length of incubation including the choice of media (e.g., yeasts on chromogenic [48 hours] versus routine media [five to seven days]) and type of fungus suspected (e.g., slow growing dimorphic systemic fungi may need eight weeks).

Once the organism has been cultured and isolated, the following approach has usually been conducted: (1) determine the morphology of the unknown fungus and determine if it is consistent with any of the groups listed in Table 18-11 or filamentous bacterium (some of the aerobic actinomycetes [e.g., *Nocardia*] resemble fungi and must be ruled out); and (2) note the rate of growth, colony and microscopic morphologies of the possible organism(s) (Table 18-12) and refer to necessary textbooks to compare descriptions, drawings, color plates, discussions of characteristics, and other test results to assist in differentiating the likely organism.[52,53] In the case of yeasts and yeast-like organisms, additional testing such as the germ tube test, biochemical testing using commercially available systems, or the urease test may allow species identification of isolates from various body sites.

Antigen Detection

Cell wall components of various invasive fungi have been used as diagnostic markers for antigen testing. Galactomannan is a polysaccharide component of the *Aspergillus* cell wall that is released by growing hyphae. A commercial ELISA (Platelia *Aspergillus* Galactomannan Test, Bio-Rad Laboratories, Marens-La-Coquette, France) is available to detect circulating galactomannan antigen in blood and has been shown to be an earlier diagnostic marker for invasive aspergillosis in neutropenic patients with hematologic malignancies.[52] The monitoring of antigen titers has also been shown to correlate with the response to antifungal therapy, patient survival, and autopsy findings in neutropenic patients. It is worth noting that commercial assay has shown cross-reactivity with *H. capsulatum*, *P. brasiliensis*, and *Cryptococcus* spp.

Several commercial kits are available for the detection of capsular galactoxylomannan (cryptococcal antigen) and are able to detect *C. neoformans* and *Cryptococcus gattii*. Latex antigen detection and enzyme immunoassay (EIA) are sensitive (93–100%) and specific (93–100%) diagnostic tests for the detection and quantitation of circulating *C. neoformans* (capsular galactoxylomannan) polysaccharide antigen in serum and CSF.[52] Antigen testing is considered to be the primary diagnostic test for screening CSF for suspected cases of cryptococcal meningitis because the India ink procedure has a low sensitivity. The combination of antigen detection test and an India ink stain of the CSF are recommended for the primary evaluations of suspected cases of cryptococcal meningitis. The reported titer determinations of the two testing methods (e.g., EIA versus latex testing) or from different commercial latex kits are not numerically similar. Thus, the same testing method and latex kit should be used to monitor serial samples for a patient. False-negative and false-positive (e.g., rheumatoid factor) results have been reported for each testing method.

EIA can be used to detect *H. capsulatum* antigen in body fluids (e.g., blood, urine, CSF or bronchoalveolar lavage [BAL] fluid). It has been recommended that the antigen screening test be validated by antibody testing (e.g., immunodiffusion [ID] and complement fixation [CF]).[52] The diagnosis of histoplasmosis should be based on a combination of diagnostic test results because antigen testing is associated with cross-reactivity to other fungal infections (e.g., *Blastomyces*, *Coccidioides*, *Paracoccidioides*) and the test sensitivity varies with disease presentation (e.g., 77% for acute pulmonary histoplasmosis, 34% for subacute pulmonary histoplasmosis, 21% for chronic pulmonary histoplasmosis, 92% for progressively disseminated histoplasmosis) and specimen type (80–95% in urine, 25–50% in CSF, 93.5% in BAL). Antigen detection is generally not used as a diagnostic tool and has a limited role for blastomycosis and coccidioidomycosis due to low levels of detection in antigenemia and antigenuria, cross-reactions, and false-positive reactions. Antigen detection tests for *H. capsulatum*, *B. dermatitidis*, and *Coccidioides* species are performed by MiraVista Diagnostics (Indianapolis, IN) on a fee-for-service basis.

Antigen detection methods and serology for *Candida* spp. in blood cultures have not been reliable in distinguishing between colonization, candidemia, and disseminated candidiasis.[52] Extreme variability has been observed in both sensitivity and specificity, making the currently available tests unreliable for establishing a diagnosis. To maximize the diagnosis of invasive candidiasis, the combination of both the mannan antigen and the antimannan antibody test (Platelia *Candida* Ag Plus, Bio-Rad Laboratories, Marens-La-Coquette, France) should be performed.

Serology

Several different methodologies for antibody testing (e.g., tube precipitin [TP], CF assays, ID, LA, and EIA) have been investigated for the detection of specific fungal pathogens.[52] Interpretation of *serology* results for most fungal infections requires knowledge of the laboratory technique used to perform the antibody testing. Serologic assays are most useful as diagnostic testing of fungal infections in the immunocompetent host because a poor antibody response is common in immunosuppressed patients resulting in a false-negative result.

The presence of antibody has assisted in the diagnosis of invasive infections such as coccidioidomycosis, histoplasmosis, and paracoccidiodomycosis.[52] The most reliable serological tests for diagnosing coccidioidomycosis have been ID and CF, where heated and unheated coccidioidin is used as the principal antigen in these tests. Serologic tests (ID, CF, and LA) for the clinical diagnosis of infections caused by *H. capsulatum* are commercially available. These tests have been the most useful in patients with chronic pulmonary or disseminated histoplasmosis. Compared to ID, the CF test is more sensitive but has more cross-reactivity and positive reactions in patients with fungal (e.g., aspergillosis, blastomycosis, candidiasis, coccidioidomycosis, paracoccidioidomycosis) and other type of infections (e.g., TB, bacterial or viral). ID and CF are the most common serologic methods used for the diagnosis of paracoccidioidomycosis. No commercial kits for either method are available (fee-for-service, Cerodex Laboratories, Washington, OK). EIA is also available; however, it should be used as a screening tool and positive results should be confirmed by another method. Finally, serology testing has been useful for the diagnosis of noninvasive diseases such as allergic bronchopulmonary aspergillosis, aspergilloma, and chronic cavitary aspergillosis.[52]

(1,3)-β-D-Glucan Detection

Commercial assays for the detection of *(1,3)-β-D-glucan*, a polysaccharide present in cell wall of common pathogenic yeasts, have been used as a panfungal diagnostic tool for invasive fungal infections such as aspergillosis, *Fusarium* infection, trichosporonosis, and candidiasis.[52] This assay has also been used to detect (1,3)-β-D-glucan from *P. jirovecii*, in both HIV-positive and HIV-negative patients. In the United States, Fungitell β-D-Glucan assay (Associates of Cape Cod Incorporated, East Falmouth, MA) serum detection test is the only available EIA for detecting (1,3)-β-D-glucan. The manufacturer's recommended guidelines for a positive (1,3)-β-D-glucan value is ≥80 pg/mL and a negative value <60 pg/mL; values between 60 and 79 are considered indeterminate (http://www.acciusa.com). False-positive results have been observed in hemodialysis patients (with cellulose membranes), patients treated with certain blood products (e.g., albumin, immunoglobulins), and patients with bacterial infections or have been exposed to glucan-containing materials (e.g., gauze). Concurrent β-lactam therapy, such as piperacillin/tazobactam or amoxicillin/clavulanate, and antitumoral polysaccharides have been associated with false-positive results. This assay is nonspecific and should be used in conjunction with clinical examination of the patient and other diagnostic tests and procedures to make a conclusive diagnosis of invasive fungal infection. This diagnostic assay is not useful for mucoraceous molds (e.g., Zygomycetes such as *Rhizopus*, *Mucor*, and *Absidia*), which do not produce (1,3)-β-D-glucan, or *Cryptococcus* species and *B. dermatitidis* because they produce only low levels of (1,3)-β-D-glucan. Limited evaluations have assessed this diagnostic assay for the detection of histoplasmosis and coccidioidomycosis.

Molecular Diagnosis

Currently, there are a limited number of molecular diagnostic tests available for the detection and identification of fungi in the clinical laboratory.[52-55] For fungal isolates grown in pure culture, NA hybridization probes, DNA sequencing, PNA-FISH probes, and laboratory-developed PCR tests are available. Molecular methods used in the direct detection and identification of fungi from patient specimens are limited to a few laboratory-developed PCR tests targeting specific fungal agents. A commercially available platform (Luminex xMAP, Luminex, Austin, TX) with PCR amplification, flow cytometry, and dual-laser system is available for high throughput and species-specific identification with user-designed (or outside vendor) probes. For laboratory-developed methods, proper validation of NA tests is needed before routine use can occur in the clinical laboratory. Clinicians will need to contact their laboratory to determine which tests are available and which molecular diagnostic tests may need to be sent to a reference laboratory. At this time, a combination of morphologic and molecular testing methods is best used for species identification.

Several probe-based assays have become commercially available for the identification of dimorphic fungi and *Candida* spp.[52,53] AccuProbe (Gen-Probe, San Diego, CA) uses hybridization to target rRNA present in a fungal culture, which is detected by a labeled single-stranded DNA probe. Three separate probes, with high sensitivity and specificity, are approved by the FDA and are available for the identification of *B. dermatitidis*, *Coccidioides immitis*, and *H. capsulatum*. The *B. dermatitidis* probe has the potential to cross-react with other fungi including *Emmonsia* species, *Paracoccidiodes brasiliensis*, and *Gymnascella* spp. In addition, the *Coccidioides* probe is unable to distinguish between species, namely *Coccidioides immitis* and *Coccidioides posadasii*.

An extensive number of evaluations have been ongoing for different PCR assays for invasive *Aspergillus* and *Candida* spp. infections. Limited and variable sensitivity and specificity have been some of the main issues restricting the routine use of this method. Additional issues that need to be addressed include specimen type, sample volume, best method of DNA extraction, target range, and definitions of positive results. Similar to other fungal infections, NA detection remains mainly as a research-based tool. However, further evaluation with standardized methodology and decreased inconsistencies between tests should allow PCR to become a promising method for detection of *Aspergillus* and *Candida* spp. Novel technologies (e.g., T2Candida Panel and automated T2Dx Instrument) are allowing rapid (e.g., three to five hours) and accurate diagnosis of invasive candidiasis directly from the patient whole blood samples (no need for blood culture and isolation of *Candida* spp.).

PNA-FISH (PNA FISH Yeast Traffic Light Probe, AdvanDx, Woburn, MA) is available for the direct identification of *Candida* spp. on blood smears from cultures that are Gram stain positive for yeasts.[53] After the Gram stain and the hybridization process is completed, *C. albicans* and *C. parapsilosis* are identified microscopically as bright green fluorescing cells, while *Candida tropicalis* fluoresces bright yellow, and *C. glabrata* and *C. krusei* fluoresce bright red. Other yeasts do not fluoresce. The colors of the light probes also provide an indication about the potential use of fluconazole in these patients because *C. albicans* and *C. parapsilosis* are generally susceptible to fluconazole (green light for go), *C. glabrata* can be resistant to fluconazole and *C. krusei* is intrinsically resistant to fluconazole (red light for stop). The yellow signal produced by *C. tropicalis* indicates that caution should be used because fluconazole susceptibility is variable for this organism. This method has a significant impact over traditional identification methods, which could take up to three or more days for identification of *Candida* spp., as well as guiding the most effective antifungal drug therapy.

MALDI-TOF Mass Spectrometry

Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) *mass spectrometry* (MS) is ideal for genus and species identification and has the potential for accurate strain typing and identification for fungi, bacteria, and mycobacterium.[57,58] This technology is a rapid and accurate method for identifying yeasts and molds recovered on culture media and using sample preparation for MALDI-TOF MS.[52-54,57,58] Reports on the use of MALDI-TOF MS for routine rapid identification have focused on clinically important yeasts (e.g., *Candida* spp., *C. neoformans–C. gattii* spp.) and dermatophyte species (e.g., *Neoscytalidium* spp., *Trichophyton*, *Microsporum*, *Epidermophyton*, and *Arthroderma*). Filamentous fungi and molds (e.g., *Aspergillus* spp., *Fusarium* spp., *Pseudallescheria–Scedosporium* Complex, *Penicillium* spp., *Lichtheimia* spp.) have been more difficult because of different developmental forms on agar media and the influences of the phenotype. MALDI-TOF MS is likely to become the primary diagnostic method for rapid identification of fungus isolates in the clinical microbiology laboratory. However, its use for fungal identification has moved at a slower pace than the current use of MALDI-TOF MS for bacterial identification

The advantages of the MALDI-TOF MS for fungal identification is the low cost of materials (a few cents) for each organism identification and the rapidity to results (approximately 11 minutes if just one isolate is tested; 2.5 minutes per isolate in a batch of 96 isolates, with the average time per isolate in published reports being four to six minutes). However, current limitations include the initial costs of instrumentation for the system, lack of sample preparation techniques, and inadequate fungal spectra in the database and software of commercial systems (e.g., Bruker and Shimadzu). Expansion of database libraries and developments in sample preparation are rapidly occurring to establish validated and routine procedures for a large number of clinically important fungal strains and species. Evidence from several studies also suggests that MALDI-TOF MS could be developed for performing rapid antifungal susceptibility testing (e.g., caspofungin for isolates of *Candida* spp.). Finally, MALDI-TOF MS is being investigated for epidemiological testing of fungal isolates for outbreak investigations.

Antifungal Susceptibility Testing

The importance of *antifungal susceptibility testing* has become increasingly recognized as a useful component in the treatment optimization of invasive infections caused by *Candida* spp. because of the increasing number of available antifungal agents, emerging resistance issues to standard therapy, and the changing epidemiology of invasive fungal disease. Obtaining antifungal susceptibility testing is particularly important when azole-resistant isolate is suspected or when failure to respond to antifungal therapy has occurred.

The CLSI and European Committee on Antimicrobial Susceptibility Testing (EUCAST) has developed standardized reference methods for macrodilution and microdilution susceptibility testing of yeasts and molds, as well as disk diffusion methods of yeasts and nondermatophyte filamentous fungi.[51,59,60] The commercial availability of simplified and automated testing methods (e.g., Etest strip; Vitek 2; Sensititre YeastOne) consistent with CLSI reference methods is allowing an increasing number of clinical laboratories to routinely perform antifungal susceptibility testing.

Interpretive MIC breakpoints based on CLSI- and EUCAST-recommended in vitro susceptibility testing methods have been recommended for *Candida* spp.[59-61] Comprehensive reviews regarding the microbiological, molecular, pharmacokinetic-pharmacodynamic, and clinical antifungal data for *Candida* spp. provides species-specific interpretive clinical breakpoints for azole agents and the echinocandins (**Table 18-15**).[59-61] These data have been used to establish epidemiologic cutoff values, detect emerging resistance among *Candida* spp., and harmonize antifungal susceptibility testing standards by CLSI and EUCAST.[59-61] Interpretive breakpoint criteria for amphotericin B and triazole agents against *Aspergillus* spp. have been reported; other fungal pathogens remain to be standardized.[60]

VIRUSES

There are approximately 650 viruses that are known to cause infection in humans and other vertebrate animals.[62] The three major properties that classify viruses into families include (1) the NA core (either DNA or RNA, but not both); (2) whether the viral NA is single-stranded or double-stranded; and (3) the presence or absence of a lipoprotein envelope (**Table 18-16** and **Table 18-17**).[62-64] Viruses also differ based on their genome topology (e.g., linear, circular, single versus multiple segments). Virus families can be further categorized on the basis of morphology (e.g., size, shape, and substructure), mode of replication, and molecular and genomic characteristics. The most recent information on the rapidly changing classification and

TABLE 18-15. Species-Specific Breakpoints for *In Vitro* Susceptibility Testing of *Candida* spp. According to CLSI and EUCAST[58]

ANTIFUNGAL AGENT	*CANDIDA* SPP.	MIC BREAKPOINT (MG/L)			
		CLSI		EUCAST	
		S	R	S	R
Anidulafungin	*C. albicans*	≤0.25	>0.5	≤0.03	>0.03
	C. krusei, *C. tropicalis*	≤0.25	>0.5	≤0.06	>0.06
	C. parapsilosis[a]	≤2	>4	≤0.002	>4
	C. glabrata	≤0.125	>0.25	≤0.06	>0.06
Micafungin	*C. albicans*	≤0.25	>0.5	≤0.016	>0.03
	C. krusei, *C. tropicalis*	≤0.25	>0.5		
	C. parapsilosis[a]	≤2	>4	≤0.002	>2
	C. glabrata	≤0.06	>0.125	≤0.03	>0.03
Fluconazole	*C. albicans,* *C. tropicalis,* *C. parapsilosis*	≤2	>4	≤2	>4
	C. glabrata[a]	SDD[b]: ≤32	SDD[b]: >32	≤0.002	>32
Posaconazole	*C. albicans,* *C. tropicalis,* *C. parapsilosis*			≤0.06	>0.06
Voriconazole	*C. albicans,* *C. tropicalis,* *C. parapsilosis*	≤0.125	>0.5	≤0.125	>0.125
	C. krusei	≤0.5	>1		

CLSI = Clinical and Laboratory Standards Institute; EUCAST = European Committee for Antimicrobial Susceptibility Testing; S = susceptible; R = resistant.

[a] The wild-type populations of *C. parapsilosis* to anidulafungin and micafungin, and *C. glabrata* to fluconazole, are classified as intermediate (I) category (values between S and R) to accommodate use of these agents in some clinical situations.

[b] The wild-type population of *C. glabrata* is classified by CLSI as susceptible dose-dependent (SDD) to fluconazole to accommodate use of fluconazole at higher doses in some clinical situations.

taxonomy of viruses can be obtained from the website database (www.ictvonline.org) that has been established by The International Committee on Taxonomy of Viruses (ICTV). The 2013 ICTV report now recognizes five hierarchical ranks consisting of seven orders, 103 families, 22 subfamilies, 455 genera, and 2827 species of viruses; however, over 3000 viruses remain unclassified.

The Identification of Viruses

The ability to detect and accurately identify viruses in the clinical laboratory has increased during the last 30 years as a result of wider applicability of diagnostic laboratory techniques with increased sensitivity and decreased turnaround time, the availability of newer reagents and rapid commercial diagnostic kits, and the addition of new antiviral drugs for specific viral infections.[64-67] In addition, several NA amplification techniques (NAATs), specifically PCR and real-time PCR, are allowing routine clinical laboratories to provide virology services for the increasing frequency of infectious diseases that depend on rapid viral diagnosis.[64,66,67]

It is important to note that all diagnostic tests for the identification of viruses are not available at each institution, and the clinician will need to establish a relationship with the laboratory that will be performing viral testing. In certain clinical situations, samples may need to be sent out for diagnostic testing at either large reference or public health laboratories because they are able to provide the necessary methods that are difficult or impossible to routinely perform in the clinical virology laboratory. In addition, certain viruses (e.g., arboviruses, arenaviruses, filoviruses, Variola virus, and rabies virus) require testing at biosafety level (BSL) 3 or 4 facilities and are often sent to the Centers for Disease Control and Prevention (CDC) or the CDC's Division of Vector-Borne Infectious Diseases.[64]

The ability to accurately diagnose a viral infection is highly dependent on appropriate selection, timing, collection, and handling of biological specimens.[63,65] In general, the highest titers of viruses are present early in the course of illness and decrease as the duration of illness increases. Therefore, it is very important to collect specimens for the detection of viruses early in the course of an infection. In most cases, identification of viruses is a specimen-driven process. Because collection procedures are highly dependent on viruses being suspected, attention needs to be taken regarding collection containers and devices as well as transport systems (e.g., whether a viral

TABLE 18-16. Characteristics and Laboratory Diagnosis of Selected DNA Viruses of Medical Importance to Humans[62-67]

FAMILY	NATURE	ENVELOPE	SHAPE	NUCLEOCAPSID, SYMMETRY	EXAMPLES OF SPECIES COMMONLY INFECTING HUMANS	METHODS COMMONLY USED FOR DETECTION OF VIRUS[a]
Adenoviridae	dsDNA, linear	No	Isometric	Icosahedral	Human mastadenovirus A to G	NAAT, culture, and IFA widely used for respiratory specimens NAAT used to monitor viral load in compromised hosts Rapid antigen assays used for ocular and enteric adenoviruses
Anelloviridae	ssDNA (-), circular	No	Isometric	Icosahedral	Torque teno virus 1	NAAT, Cell culture
Hepadnaviridae	dsDNA, reverse transcribing circular	Yes	Spherical	Icosahedral	HBV	IA, NAAT, serology NAAT used to monitor therapy and determine genotype
Herpesviridae	dsDNA, linear	Yes	Spherical	Icosahedral	Human herpesvirus 1 and 2 (HSV-1 HSV-2); macacine herpesvirus 1 (B virus)	NAAT, serology, IFA, cell cultures
					Human herpesvirus 3 (VZV)	NAAT, IFA, serology, cell culture
					Human herpesvirus 4 (EBV)	Serology, NAAT, histology (lymphoid disorders)
					Human herpesvirus 5 (human CMV)	Cell culture, NAAT and pp65 antigemia (assess risk of disease and response to therapy), serology (to determine prior infection)
					Human herpesvirus 6 (HHV-6A and 6B) and 7 (HHV-7)	NAAT, serology (parotitis)
					Human herpesvirus 8 (Kaposi's sarcoma–associated herpesvirus)	Serology, histology, NAAT
Papillomaviridae	dsDNA, circular	No	Isometric	Icosahedral	Human papillomavirus (HPV 1, 4, 5, 32, 41)	NAAT, cytopathology
Parvoviridae	ssDNA, linear	No	Isometric	Icosahedral	Human parvovirus B19 (exanthema in children)	NAAT, serology
Polyomaviridae	dsDNA, circular	No	Isometric	Icosahedral	JC polyomavirus (JCV) BK polyomavirus (BKV)	NAAT, histology NAAT, cytology
Poxviridae	dsDNA, linear	Yes	Brick-shaped or oval	Complex	Variola virus (Smallpox virus); Vaccinia virus; Cowpox virus; Monkeypox virus Molluscum contagiosum virus Orf virus Yaba monkey tumor virus	NAAT. EM, serology, cell culture[b]

(-) = negative stranded; CMV = cytomegalovirus; EBV = Epstein-Barr virus; EM = electron microscopy; dsDNA, double-stranded DNA; HAV = hepatitis A virus; HBV = hepatitis B virus; HPV = human papillomavirus; HSV = herpes simplex virus; IA = immunoassay; NAAT = nucleic acid amplification techniques; ssDNA = single-stranded DNA; VZV = varicella-zoster virus.

[a] Commonly used methods in clinical laboratories: immunoassays including immunofluorescence assay (IFA), enzyme-linked immunosorbent assay (ELISA), and immunochromatographic assay (ICA).

[b] The isolation of some pathogens (e.g., smallpox) requires biosafety level (BSL) 3 or 4 facilities, usually only in specialized centers collaborating with World Health Organization. The isolation of Vaccinia virus requires BSL-2 (grows readily in cell culture).

TABLE 18-17. Characteristics and Laboratory Diagnosis of Selected RNA Viruses of Medical Importance to Humans[62-67]

FAMILY	NATURE	ENVELOPE	SHAPE	NUCLEOCAPSID, SYMMETRY	EXAMPLES OF SPECIES INFECTING HUMANS	METHODS COMMONLY USED FOR DETECTION OF VIRUS[a]
Arenaviridae	ssRNA , linear	Yes	Spherical, pleomorphic	Helical	Lassa virus	NAAT, cell culture; (BSL 4)
					Lymphocytic choriomeningitis virus (LCMV)	Serology, NA
					Guanarito virus	Serology; cell culture (BSL 4)
					Junin virus	Serology; cell culture (BSL 4)
					Machupo virus	Serology; cell culture (BSL 4)
					Sabia virus	Serology; cell culture (BSL 4)
Astroviridae	ssRNA (+), linear	No	Isometric	Icosahedral	Human astrovirus	EM, NAAT, IA
Bornaviridae	ssRNA (-), linear	Yes	Spherical	Helical	Borna disease virus	Serology, IA, NAAT
Bunyaviridae	ssRNA, linear	Yes	Spherical, pleomorphic	Helical	California encephalitis virus	Serology, antibody detection in CSF
					La Crosse virus	Serology, IFA
					Hantaan virus	Serology, EM, NA
Caliciviridae	ssRNA (+), linear	No	Isometric	Icosahedral	Norovirus	NAAT, IA
					Sapovirus	NAAT, EM, IA
Coronaviridae	ssRNA (+), linear	Yes	Spherical, pleomorphic	Helical	Human coronavirus 229E, NL63, HKU1	NAAT, serology
Deltavirus	ssRNA (-), circular	Yes	Spherical	Helical	HDV	Testing confined to reference laboratories
						Serology, histology
Filoviridae	ssRNA (-), linear	Yes	Filamentous, pleomorphic	Helical	Bundibugyo ebolavirus; Sudan ebolavirus; Tai Forest ebolavirus; Zaire ebolavirus	Testing confined to reference laboratories
						IA, NAAT, EM, cell culture (BSL 4)
					Marburg marburgvirus	Testing confined to reference laboratories
						EM, cell culture (BSL 4)
Flaviviridae	ssRNA (+), linear	Yes	Spherical	Icosahedral	Tick-borne encephalitis virus	Testing confined to reference laboratories.
						Serology, antibody detection (CSF)
					Dengue virus	Testing confined to reference laboratories
						Serology, NA, antibody detection (CSF)
					Japanese encephalitis virus	Serology, antibody detection (CSF)

(*continued*)

TABLE 18-17. Characteristics and Laboratory Diagnosis of Selected RNA Viruses of Medical Importance to Humans[62-67], cont'd

FAMILY	NATURE	ENVELOPE	SHAPE	NUCLEOCAPSID, SYMMETRY	EXAMPLES OF SPECIES INFECTING HUMANS	METHODS COMMONLY USED FOR DETECTION OF VIRUS[a]
					Murray Valley encephalitis virus	Testing confined to reference laboratories Serology, antibody detection (CSF), NA
					St. Louis encephalitis virus	Testing confined to reference laboratories Serology, antibody detection (CSF)
					West Nile virus	Testing confined to reference laboratories Serology, antibody detection (CSF)
					Yellow fever virus	Testing confined to reference laboratories Serology, NA, histology
					HCV	Serology, NAAT
Hepeviridae	ssRNA (+), linear	No	Isometric	Icosahedral	HEV	Serology, NAAT (transplant patients but not commercially available)
Orthomyxoviridae	ssRNA (-), linear	Yes	Pleomorphic	Helical	Influenza virus A, B, C	NAAT, IA, IFA, rapid culture
Paramyxoviridae	ssRNA (-), linear	Yes	Pleomorphic	Helical	Mumps virus	Serology, cell culture, NAAT (for outbreaks)
					Measles virus	Serology, NAAT (public health laboratories)
					Parainfluenza virus 1,2,3,4	NAAT, cell culture, IFA
					Human RSV	NAAT, IFA, cell culture, serology (epidemiological studies)
					Human metapneumovirus	NAAT, IA
Picobirnaviridae	dsRNA, linear	No	Isometric	Icosahedral	Human picobirnavirus	NAAT, cell culture
Picornaviridae	ssRNA (+), linear	No	Isometric	Icosahedral	Human enterovirus A, B, C, D	NAAT, cell culture
					Human rhinovirus A, B, C	NAAT, cell culture (usually not necessary)
					HAV	Serology
					Human parechovirus	NAAT, cell culture
Reoviridae	dsRNA, linear	No	Isometric	Icosahedral	Rotavirus A, B, C	Direct antigen detection (IA, LM), new NAAT gastroenteritis panels include rotavirus, EM

TABLE 18-17. Characteristics and Laboratory Diagnosis of Selected RNA Viruses of Medical Importance to Humans[62-67], cont'd

FAMILY	NATURE	ENVELOPE	SHAPE	NUCLEOCAPSID, SYMMETRY	EXAMPLES OF SPECIES INFECTING HUMANS	METHODS COMMONLY USED FOR DETECTION OF VIRUS[a]
Retroviridae	ssRNA (+), linear	Yes	Spherical	Icosahedral	Human immunodeficiency viruses types 1 (HIV-1) and 2 (HIV-2)	Serology, antigen/antibody combination, quantitative RNA tests to guide therapy and monitor response Proviral DNA tests useful for neonatal infections
					Primate T-lymphotropic viruses, (HTLV-1 and HTLV-2)	Serology, NAAT
					Simian foamy virus	Serology, NAAT
Rhabdoviridae	ssRNA (-), linear	Yes	Bullet shaped	Helical	Rabies virus	For human rabies, testing done at CDC NAAT, cell culture, IFA, serology
Togaviridae	ssRNA (+), linear	Yes	Spherical	Icosahedral	Arboviruses including *Alphavirus* genus, containing ~25 viruses	Serology, cell culture Isolation of some agents may require BSL3 or BSL4 facilities
					Rubella virus	Serology, NAAT (public health laboratories), cell culture (prodromal period to 4 days postrash)

(+) = positive stranded; (-) = negative stranded; BSL = biosafety level; CDC = Centers for Disease Control and Prevention; CSF = cerebrospinal fluid; dsRNA = double-stranded RNA; EM = electron microscopy; HAV = hepatitis A virus; HCV = hepatitis C virus; HDV = hepatitis D virus; HEV = hepatitis E virus; HIV = human immunodeficiency virus; IA = immunoassay; LA = latex agglutination; NAAT = nucleic acid amplification techniques; ssRNA = single-stranded RNA; RSV = respiratory syncytial virus.

[a] Commonly used methods in clinical laboratories: Immunoassays, including immunofluorescence assay (IFA); enzyme-linked immunosorbent assay (ELISA), and immunochromatographic assay (ICA).

transport medium is needed). The different types of clinical specimens that can be collected for viral culture and antigen detection include respiratory specimens (e.g., nasopharyngeal swabs, aspirates, and washes; throat swabs; BAL and bronchial washes), blood, bone marrow, CSF, stool, biopsy tissue, urine, ocular specimens, vesicles and other skin lesions, and amniotic fluid. In addition, specimens for molecular diagnostic testing (e.g., PCR and other nucleic amplification techniques) must be obtained following specific guidelines so that the stability and amplifiability of the NAs are ensured.

Once the sample is collected, it should be promptly transported to the laboratory in a sterile, leak-proof container using the appropriate viral transport media to maximize viral recovery. Every effort should be made to prevent delay between the time of specimen collection and its arrival to the laboratory. When delays are expected, viral samples should be refrigerated at 4 °C or frozen at –70 °C. Subsequently, the laboratory will need to follow specific processing procedures for each specimen and the different diagnostic viral test methodologies.

The laboratory techniques used in the diagnosis of viral infections include cell culture, cytology and histology, electron microscopy (EM), antigen detection, NAATs, and serologic testing.[64-67] The choice of test(s) will vary depending on the clinical syndrome or disease, virus(es) involved, patient characteristics, collection site, purposes of the test (e.g., screening, diagnosis, confirmation or monitoring), time to result, laboratory capabilities/staff expertise, and cost. The following section, as well as Tables 18-16 and 18-17, provide a brief summary of the common methods currently used in diagnostic testing of common viruses.[64-67] For more detailed information, the reader is referred to current published literature, standard reference books, and the latest edition of reference manuals (e.g., *Manual of Clinical Microbiology*, American Society for Microbiology Press).

Cell Culture

The use of *cell culture* rapidly expanded the knowledge about the epidemiology, clinical characteristics, and diagnosis of common viral infections in the 1950s and 1960s. Subsequently, the use of cell cultures to isolate a virus became the gold standard method for the diagnosis of viral infections in most clinical virology laboratories during the past 20 years.[64-67] However, rapid and accurate serology and molecular methodologies have become a cornerstones for virus detection and identification in clinical laboratories during the past few years, resulting in a decline in the use and prominence of cell cultures in larger academic medical centers and tertiary-care facilities. Despite these changes in routine diagnostic virology, viral cultures have an important role for discovery of new viruses, identification of variants of known viruses, detection of drug-resistant viruses, detection of viruses in special patient populations (e.g., immunocompromised patients), and for research and development of antiviral drugs and vaccines.[67]

The advantages of cell culture include good specificity and sensitivity, the capability of detecting multiple viruses if present, and the cultivation of the virus for further laboratory testing (e.g., susceptibility testing, serologic strain typing), if needed. Cell cultures can be useful when combined with highly specific monoclonal antibodies (MAbs), if the cost of other testing methods is greater than cell cultures or when the clinical laboratory does not have the ability and equipment to perform molecular detection methods. The disadvantages of cell culture include the long time needed for the detection of viruses using conventional cell culture (e.g., days to weeks), the need for cell culture facilities, the expense of performing cell culture, and the methodology is not applicable to all viruses (e.g., some viruses are "noncultivable", i.e., Group C rhinovirus). This greater technical demand that cell cultures place on a clinical and diagnostic virology laboratory is being replaced by rapid and sensitive antigen screening assays and NAATs.

There are several different types of cell culture that are available to grow clinically important viruses.[64-67] Each virus requires a predefined cell line, which is established once a cell culture has been subcultured in vitro. The different types of cell lines can be divided into three categories: primary, diploid (also called *low passage cell lines*), and heteroploid. Primary cell lines (e.g., rhesus monkey kidney [RhMK] cells or human amnion cells) are prepared from animal or human tissues and can withstand only one or two passages until the cells die. Diploid cell lines are usually derived from fetal or newborn cells (e.g., human embryonic lung fibroblast lines such as WI-38 or MRC-5) and can undergo 20 to 50 passages before cells are unable to survive. Continuous cell lines can undergo an indefinite number of passages without reducing the sensitivity to virus infection. Heteroploid cell lines are characteristically derived from human or animal cancers (e.g., human epidermoid lung carcinoma [HEp-2, HeLa]) or are cells transformed in vitro (e.g., LLC-MK_2). Heteroploid cell lines can also include genetically engineered cells (e.g., ELVIS cell mixture for the detection of HSV types 1 and 2). Most specimens are inoculated onto two or more cell lines (e.g., RhMK, MRC-5, HEp-2) based on the most likely viruses associated with the type of clinical specimen that was submitted.

The growth of a virus from a clinical specimen provides direct evidence that the patient was infected with a virus. The main method for detecting growth from the cell culture method is by microscopic examination of the unstained cell cultured monolayers for morphological changes or cytopathic effect (CPE).[64-67] The characteristics of the CPE (e.g., which cell culture types were affected; what is the resultant shape of the cells; whether the effect is focal or diffuse; the time of its appearance and progression) can be used for primary and definitive identification of the virus. Subsequently, fluorescent antibody (FA) staining of cells with virus-specific MAbs harvested from the culture is often used to confirm the identification of the virus. Molecular or ancillary traditional testing can alternatively be used for viral identification.

Some viruses, such as influenza, parainfluenza, and mumps virus, will grow in cell cultures without producing CPE so that other methods are used to identify and detect these viruses, including hemadsorption and interference.[65-67] Hemadsorption is used to detect these viruses, which can grow rapidly and reach high titers in cell cultures without producing CPE. Hemadsorption involves the removal of the culture medium from the inoculated cell culture, adding a suspension of erythrocytes and examining for hemadsorption with a low-power microscope as manifested by adherence of the red cells to the cell culture monolayer due to the presence of a hemadsorbing virus. Used to detect viruses such as rubella, interference involves growing a virus that yields a cell culture resistant to other viruses (to which it is normally susceptible). The viruses that produce hemadsorption or interference subsequently can be identified by staining with virus-specific monoclonal antibodies or antiserum.

Shell vial cultures with centrifugation and pre-CPE detection are used to decrease the amount of time required to grow a virus by conventional cell cultures.[64-67] This technique makes use of cells grown on microscope coverslips that are placed within shell vials and covered with culture media. After cultures are incubated for one to three days, FA staining is performed on the cells on the coverslips to recognize an antigen in the nucleus of infected cells. Shell vial cultures have been commonly applied for the detection of cytomegalovirus (CMV), HSV, varicella-zoster virus (VZV), enteroviruses, and the human respiratory viruses. Centrifugation-enhanced rapid cell cultures can also be used with co-cultivated cells (e.g., mixture of two cell lines together) or genetically engineered cells (e.g., ELVIS [enzyme-linked virus-inducible system], BGMK-hDAF [buffalo green monkey kidney cell line]) for the rapid identification (e.g., 16–72 hours) and blind staining of multiple viruses from a single shell vial or tray well.[64,65,66]

Molecular Diagnosis

The detection of specific viral NAs by molecular diagnostic techniques is revolutionizing the field of diagnostic virology.[64-68] NA amplification techniques (NAATs) have become the "gold standard" in clinical virology laboratories and replacing older techniques such as cell cultures for detecting clinically significant viruses. The techniques used in viral NA detection include direct hybridization assays, target (template) amplification (e.g., PCR, self-sustained sequence replication method, strand displacement amplification), and signal amplification (e.g., branched-chain DNA [bDNA] assay and hybrid capture assay). Among these, PCR has been the most important technique in diagnostic virology because of its versatility in detecting DNA or RNA, as well as being able to provide qualitative and quantitative information on specific viral NAs.

The use of NA detection has become the standard of care (e.g., HCV and HIV) or the test of choice for routine diagnosis of many viral infections (e.g., bocaviruses, HSV central nervous system (CNS) infections, human HVS 6 and 7, human metapneumovirus, human papillomavirus [HPV]).[64,68] The FDA has cleared or approved commercial molecular detection assays, several viruses including hepatitis B and C viruses (HBV, HCV), HIV, HSV, CMV, adenovirus, avian flu, enteroviruses, influenza, and HPV. An FDA-approved simple multiplex PCR test (e.g., xTAG Respiratory Viral Panel) is also available for rapidly screening common respiratory viruses (e.g., RSV, influenza A and B, adenovirus) or subtypes. In addition, FDA-approved high-throughput tests are available for the detection of common GI viruses. An up-to-date listing of FDA-approved/cleared molecular diagnostics tests is available on the website of the Association of Molecular Pathology (www.amp.org/FDATable/).

The advantages of viral NA detection methods include the rapidity of results (e.g., hours for real-time PCR and one to several days for other methods), maximal sensitivity for virus-specific detection and identification, adequate to excellent specificity, increasing availability of commercial assays, the ability to detect viruses that are difficult to culture, and the ability to detect NAs without viable virus present in the clinical specimen. Currently, equipment and reagents costs are the major barrier to implementing molecular testing. Molecular assays have become the standard of care for diagnosing viral infections as well as monitoring antiviral therapy and patient outcomes.[64,66,68]

Cytology and Histology

Cytopathologic effects (CPEs) on cells are produced by many viruses. Cytologic examination can be performed on smears prepared from samples that are applied to a microscope slide or "touch preps" of unfixed tissues.[65,68] Cytologic findings are suggestive of a viral infection and provide identification of cell morphologies (e.g., "owl's eye" nuclear inclusions consistent with CMV), cell lysis, or other cell changes (e.g., vacuolation, syncytia, inclusion bodies). The specific virus cannot be identified unless virus-specific immunostaining techniques are used. Applications of *cytology* to viral diagnosis include the Tzanck smear with Giemsa reagent for demonstrating the presence of HSV or VZV infection, Papanicolaou staining of cells obtained from the uterine cervix (Pap smear) for providing evidence of HPV infection, and cytologic staining of urinary sediments for screening the presence of either CMV or polyomaviruses JCV and BKV.

Similar to cytology, histologic examination of tissue provides evidence to suggest a group of viruses that may be causing infection, but it does not identify a specific virus.[65,66.68] Despite this shortcoming, histopathology has been useful in differentiating between asymptomatic viral shedding and clinically important infections of CMV and has been used for the diagnosis of CMV infections in tissue samples obtained from biopsy or at autopsy. In addition, detection of specific viral antigens by immunohistochemistry and detection of specific viral NAs by in situ hybridization (ISH) or PCR has allowed specific viruses to be identified by histopathology.

Electron Microscopy

Viruses are the smallest infectious pathogens that range in diameter from 18–300 nm.[65,68] Direct visualization of a virus with a light microscope can be performed only on pathogens with a diameter >200 nm. *Electron microscopy* (EM) allows visualization of characteristic viral morphology, and unlike direct detection or molecular methodologies, is capable of detecting the distinctive appearances of multiple viruses, if present.[65] EM is considered the most useful routine test for poxviruses.[66] Diagnostic virology laboratories also commonly use EM for detection of viruses that are not detected with cell cultures or other methods (e.g., gastroenteritis viruses such as noroviruses, coronaviruses, astroviruses, enteric adenovirus, and calicivirus).[64,66,68]

Several techniques have been incorporated to allow the visualization of viruses with EM from various types of clinical specimens. Negative staining is a technique for identification of viruses in fluid samples, stool samples, and blister fluid. Thin sectioning can be performed on tissue samples that have been fixed with specific fixatives for EM study, and it can be used to visualize the herpesviruses, respiratory viruses, and rabies virus.

More sensitive methods are replacing the routine use of EM for detecting clinically significant viruses.[64,66] The advantages of EM include its economical, quick (e.g., same day), adaptable, and straightforward approach for detecting viruses. The major disadvantages of EM include poor sensitivity, initial equipment expenses, and the need of highly skilled laboratory staff.

Direct Antigen Detection

Antigen detection methods involve the use of virus-specific antibodies directed toward viral antigens in a clinical specimen.[65,68] Examples of viruses that can be identified by *direct antigen detection* include RSV, influenza virus, parainfluenza virus, adenovirus, HSV, VZV, CMV, rotavirus, HBV, and measles virus.[64,65,66,68] The advantages of direct antigen detection include the rapidity of diagnosis (e.g., hours to one day), the usefulness for the identification of viruses that are difficult to culture, and the detection of viral specific antigens even if viable virus is not present in the clinical specimen. The disadvantages include the potential for false-positive and false-negative results, the difficulty of performing batch testing, and the lack of sensitivity necessary for diagnostic applications for all viruses (e.g., not applicable for rhinoviruses because there are more than 90 serotypes and cross-reacting antibodies).

The techniques commonly used for antigen detection include immunofluorescence assay (IFA; direct and indirect), EIA (including ELISA), chemiluminescent and fluorescence-based immunoassay, and particle agglutination assays. Several membrane immunochromatographic assays (ICAs; dipstick tests) are available as influenza diagnostic tests (e.g., Directigen Flu A or A+B Test, QuickVue influenza). These viral antigen tests have become simple to use and allow rapid detection (within minutes) of specific antigens from a single specimen in the clinical laboratory and at the point of care (e.g., outpatient facilities, physician offices, patient bedside). Rapid influenza diagnostic tests (RIDTs) have high specificity but moderate and variable sensitivity (higher in children and for detecting influenza A). The need for improved sensitivity of RIDTs was also observed during the 2009 pandemic of H1N1 influenza.

Serology

Serologic tests are designed to detect an antibody response in serum samples after exposure to viral antigens has occurred.[65,68] The major uses of *serology* for the detection of viral infections include the demonstration of immunity or exposure to a virus, the diagnosis of postinfectious sequelae, and the screening of blood products. In several clinical situations, serologic testing remains the primary means for the laboratory diagnosis of viruses that are difficult to culture or detect by direct methods (e.g., rubella virus, Epstein-Barr virus [EBV], hepatitis viruses, HIV, arboviruses).[64,66] Serologic testing may also serve as a supportive or adjunctive role in clinical situations where viral cultures or direct detection methods are available.

For viral infections, serologic testing can (1) identify the virus; (2) distinguish the strain or serotype; (3) differentiate between primary infection and reinfection; and (4) determine if the infection is in an acute or convalescent phase. Virus-specific immunoglobulin antibodies (e.g., IgM or IgG) are produced during the time course of a viral infection. In general, virus-specific IgM is detected in serum sooner than virus-specific IgG. The results measure the relative concentration of antibody in the body as a titer, with the titer representing the lowest antibody concentration (or inverse of the greatest dilution; a dilution of 1:128 is expressed as a titer of 128) that demonstrates activity in a patient's serum. The exact value for a titer varies with each testing method, the specific virus involved, the timing of specimen collection, and the presence of active disease.

For most viral infections, virus-specific IgM can be detected as soon as three to seven days after the onset of infection. The presence of virus-specific IgM in a single serum sample shortly after the onset of symptoms (acute phase) is usually indicative of a very recent or current primary infection. Titers of virus-specific IgM usually decline to near undetectable amounts within one to four months after the onset of infection. Virus-specific IgG can be detected during the acute phase of infection (e.g., one to two weeks) and will continue to increase for several months before reaching a maximal titer. Thereafter, the IgG titer will decline, but it usually remains detectable in serum for the remainder of a person's life. Seroconversion has occurred when at least a fourfold increase in IgG titer has occurred between serum samples collected in the acute and convalescent (two to four weeks afterward) phases. The presence of virus-specific IgG is also indicative of a past infection.

Serologic tests are also used to assess the immunity or exposure to a virus. The presence of antibody can detect which patients have been previously infected by or vaccinated for a specific virus. For example, a positive result (presence of antibody) for rubella in a woman of childbearing age implies that congenital infection will not occur during subsequent pregnancies. A negative result (absence of antibody) implies susceptibility to infection, and the woman should receive rubella vaccination as a preventative measure if she is not pregnant. Some other examples of viruses for which serologic determination of immune status is useful include hepatitis A and B (HAV, HBV), measles, mumps, parvovirus B19, and VZV.[65,68]

The techniques commonly used for serologic assays include CF, EIA, IFA, anticomplement immunofluorescence, and Western immunoblotting. In the diagnosis of certain viral syndromes (e.g., CNS infections), a serology panel may be helpful so that a battery of antigens is tested for antibody to several viruses. The advantages of viral serology include the assessment of immunity or response of a virus isolated from a nonsterile site, serum specimens are easy to obtain and store, and it can be used to identify viruses that are difficult to culture or detect by immunoassay. The disadvantages include the time to results (e.g., few days to weeks), the potential for cross-reactions between different viruses, and the need for both acute and convalescent specimens.

Antiviral Susceptibility Testing

The emergence of drug-resistant strains of viruses to antiviral agents is an increasing problem, especially in immunocompromised hosts. Unlike antibiotics, in vitro susceptibility testing of viruses has not been routinely available. The major variables that have limited the standardization of antiviral susceptibility testing include cell lines, inoculums titer, incubation period, testing range of antiviral drug concentrations, reference strains, assay methodology, and criteria, calculation, and interpretation of end-points.[69]

Susceptibility testing has been performed by phenotypic and genotypic assays for HIV-1 and -2, HBV, HCV, HSV, VZV, CMV, and influenza viruses.[69] The CLSI has published an approved standard for phenotypic susceptibility testing of HSV.[70] This standard outlines the use of a plaque reduction assay and denotes resistance to acyclovir and foscarnet when inhibitory concentration 50% (IC_{50}) values are ≥ 2 mcg/mL and ≥ 100 mcg/mL, respectively. Proposed guidelines for antiviral susceptibility results of HSV, CMV, VZV, and influenza A and B viruses for various phenotypic testing methods and antiviral agents have also been outlined.[69] Interpretation of these values must be carefully made in conjunction with the clinical response of the individual patient. Additional consensus documents and further standardization of phenotypic and genotypic assays for antiviral susceptibility testing are needed.

HUMAN IMMUNODEFICIENCY VIRUS

Human immunodeficiency virus (HIV) is the causative agent of AIDS. The HIV virus is an enveloped, positively stranded RNA virus that belongs to the Retroviridae (retrovirus) family and

Lentivirus genus. The mature virus measures approximately 100 nm in diameter and has a characteristic conical core containing proteins, enzymes, and two identical copies of single-stranded RNA. Viral proteins within the core and the lipid envelope play a significant role in the detection, diagnosis, and treatment of HIV.[71,72] The replication process of HIV involves transcription of viral RNA into proviral DNA using the reverse transcriptase (RT) enzyme. The proviral DNA is then integrated into the host's genome using the integrase enzyme, resulting in lifelong latent infection. The virus is transmitted to humans by the exchange of blood or other body fluids containing the virus through sexual contact; exposure to contaminated blood; transfusion of contaminated blood and blood products; or via contaminated needles (e.g., intravenous drug abusers or accidental needle sticks). In addition, infants can acquire HIV from an infected mother in utero, during labor or delivery, or during breastfeeding.[71,73]

There are two distinct serotypes of HIV, namely HIV-1 and HIV-2; while HIV-1 is the most prevalent serotype of HIV infections worldwide, HIV-2 infection is most commonly distributed in Western Africa and other limited geographic locations.[71-73] Routine diagnostic testing of HIV-2 is not recommended in the United States because its prevalence is extremely low. Thus, the following discussion will focus mainly on laboratory tests used for the diagnosis and management of HIV-1 infection. However, HIV-2 testing may be indicated in persons at risk for HIV-2 infection or for those who have symptoms suggestive of HIV infection with negative or indeterminate test results for HIV-1. In addition, all blood donations in the United States are tested for both HIV-1 and HIV-2.[71-73]

Laboratory Tests for HIV-1 Infection

Several laboratory tests are available for the diagnosis and monitoring of patients with HIV-1 infection. The most common virologic testing methods include HIV-1 antibody assays, HIV-1 p24 antigen assays, DNA-PCR, plasma HIV-1 RNA (viral load) assays, and viral phenotypic and genotypic assays. In addition, the absolute number of $CD4^+$ lymphocytes and the ratio of helper ($CD4^+$) to suppressor ($CD8^+$) lymphocytes ($CD4^+$:$CD8^+$ ratios) are routinely measured to evaluate the patient's immune status and response to antiretroviral therapy, because HIV primarily infects and depletes $CD4^+$ T helper lymphocytes. Viral cultures for HIV are not typically performed beyond clinical research studies due to the labor-intensive nature of the testing methods as well as the extensive time required to obtain results.[63-65]

Laboratory tests for HIV-1 infection are clinically utilized for (1) diagnosing HIV-1 infection; (2) monitoring progression of HIV infection and the response to antiretroviral therapy; and (3) screening blood donors. The selection of these tests is highly dependent on the clinical situation, the patient population, and the specified purpose for the testing, as described in **Table 18-18**.[73-77] (**Minicase 4**.) The following section briefly reviews each of the specific tests. Detailed descriptions of the various commercial assays and their clinical applications have been recently reviewed elsewhere.[71,73,74,78]

HIV Antibody Tests

Infection with HIV affects both humoral and cell-mediated immune function. The humoral immune response results in the production of antibodies directed against HIV-specific proteins and glycoproteins. For most patients, antibodies to HIV-1 can be detected in the blood by four to eight weeks after exposure to the virus. However, it may take up to 6–12 months in some patients. There are a number of tests currently available for the detection of HIV-antibody in infected patients.

The methodology of EIA (commonly referred to as *ELISA*) is widely used as the *initial* screening test to detect HIV-specific antibodies.[71,73,74,79] Like all immunoassays, ELISA is based on the concept of antigen and antibody reaction to form a measurable precipitate. The ability of ELISA to detect HIV antibodies during earlier infection has improved over recent years. Although less specific, first-generation ELISA tests introduced in 1985 were capable of detecting HIV-1 antibodies as soon as 40 days after exposure. Second-generation ELISA, introduced in 1987, incorporated recombinant antigens that increased specificity, sensitivity, and the ability to detect antibodies as soon as 34 days after infection. With the introduction of antigens from HIV-2, and the addition of antigens from HIV-1 groups M, N, and O and group M subtypes in the 1990s, specificity and sensitivity were improved. Third-generation ELISA, introduced in the mid 1990s, were redesigned as an antigen-antibody-antigen format, which dramatically improved sensitivity and specificity, and are able to detect immunoglobulin M (IgM) and non-IgG antibodies as soon as 22 days after infection.

Fourth-generation ELISA detects the presence of both HIV-1 and HIV-2 antibodies and HIV-1 p24 antigen, which has reduced the detection period to about 15 days after infection, similar to the period of detection of p24 antigen.[73] The improved sensitivity of fourth-generation assays has led to current recommendations by the CDC for their use as initial test in the diagnosis of HIV (**Figure 18-8**).[73] Commercial fourth-generation ELISA kits utilized by most diagnostic laboratories can detect both HIV-1 and HIV-2 and have a sensitivity and specificity of >99%; however, false-positive and false-negative results can occur, particularly with previous generation assays. False-positive results have been reported with improper specimen handling (e.g., heating) and in patients with autoimmune diseases, recent influenza vaccination, acute viral infection, alcoholic liver disease, chronic renal failure requiring hemodialysis, lymphoma, hematologic malignancies, and positive rapid plasma reagin (RPR) tests due to reactivity of the antibodies used for testing. Several causes have been identified for false-negative ELISA results and include the concomitant use of immunosuppressive therapy, the presence of severe hypogammaglobulinemia, and testing for HIV infection shortly after infection [acute HIV infection] or too late in the course of HIV infection.[71,73]

The results of a fourth-generation assay are reported as reactive (positive) or nonreactive (negative). If the initial result is nonreactive, no further testing for HIV antibodies is performed and the person is considered uninfected unless they are suspected of having acute HIV infection. When the initial result

TABLE 18-18. Recommended Laboratory Tests for the Diagnosis, Monitoring, and Blood Donor Screening for HIV[73-77]

CLINICAL SITUATION	RECOMMENDED TEST(S)	COMMENTS
Diagnosis of HIV infection, including acute infection (excluding infants)	Initial fourth-generation antibody/antigen ELISA + supplemental antibody differentiation immunoassay	Only a reactive initial ELISA requires a supplemental HIV-1/HIV-2 antibody differentiation immunoassay A nonreactive initial ELISA does not require further testing An HIV RNA test may be necessary if initial testing is indeterminate
Diagnosis of HIV in infants (<18 mo of age) born to HIV-infected mother	HIV DNA PCR or plasma HIV RNA viral load	Initial testing recommended between 14 and 21 days of life, 1–2 mo, and 3–6 mo; diagnosis of HIV by two positive virologic tests HIV antibody testing not recommended due to persistence of maternal antibody
Indeterminate supplemental antibody differentiation immunoassay	Nucleic acid test for HIV-1	Only HIV-1 nucleic acid test available
Prognosis	Plasma HIV RNA viral load and $CD4^+$ T cell count	Risk of disease progression greater with HIV RNA >100,000 copies/mL
Response to antiretroviral therapy	Plasma HIV RNA viral load and $CD4^+$ T cell count	Decision to start therapy should be based on laboratory results as well as clinical findings, patient interests, adherence issues, and risks of toxicity and drug interactions
Antiretroviral drug resistance testing	Phenotypic and genotypic resistance assays	Recommended for acute and chronic HIV infection on entry into care, treatment naïve patients, pregnant patients, and cases of virologic failure (testing recommended within 4 wk of treatment discontinuation) Not recommended for patients with HIV RNA <1000 copies/mL
Blood donor screening	Fourth-generation antibody/antigen ELISA + Supplemental antibody differentiation immunoassay; plasma HIV RNA viral load	In the United States, the blood from all donors is tested for HIV-1 and HIV-2 antibodies as well as p24 antigens

DNA = deoxyribonucleic acid; ELISA = enzyme-linked immunosorbent assay; HIV = human immunodeficiency virus; RNA = ribonucleic acid; PCR = polymerase chain reaction.

MINICASE 4

Testing for HIV While on Pre-Exposure Prophylaxis

Brian W. is an HIV-negative partner in a serodiscordant relationship who is currently taking tenofovir/emtricitabine for HIV pre-exposure prophylaxis (PrEP). He reports taking PrEP regularly without missed doses and does note occasional unprotected intercourse with his HIV-positive partner. He routinely gets tested for HIV every three months but is wondering if he needs to wait that long to get tested if he has an HIV sexual exposure from his partner. He has heard that it takes several months before HIV can be detected after exposure, and he would rather know as soon as possible.

QUESTION: How soon after exposure can current HIV tests detect infection?

DISCUSSION: The current tests for diagnosing HIV infection include the fourth-generation ELISA (or EIA) and a supplemental antibody differentiation immunoassay. Because fourth-generation assays combine the detection of HIV-1 and HIV-2 antibodies as well as HIV-1 p24 antigen, they are able to detect both acute and established HIV infection. The detection of HIV-1 p24 antigen by third-generation and fourth-generation immune assays is estimated to be between 14 and 20 days after infection. Thus, this patient does not need to wait until his scheduled routine HIV test and could seek earlier testing for reassurance. Additionally, he should be counseled on the importance of condom use while taking PrEP, and on the decreased likelihood of transmission if his partner has an undetectable HIV viral load.

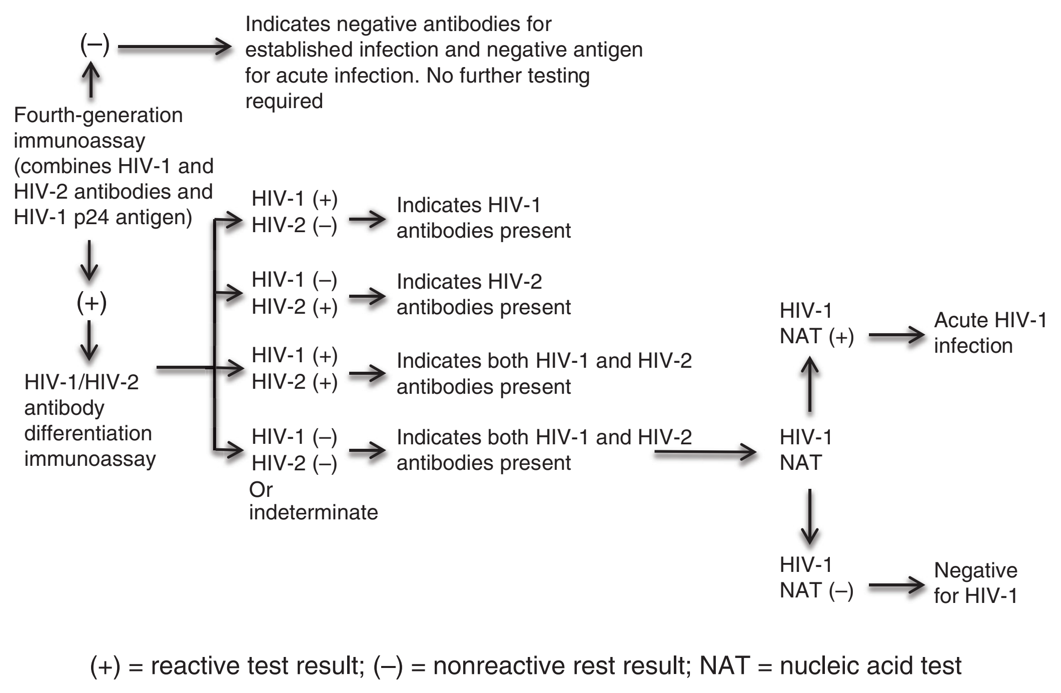

(–)
Indicates negative antibodies for established infection and negative antigen for acute infection. No further testing required
Fourth-generation immunoassay (combines HIV-1 and HIV-2 antibodies and HIV-1 p24 antigen)
(+)
HIV-1/HIV-2 antibody differentiation immunoassay
HIV-1 (+) HIV-2 (–)
Indicates HIV-1 antibodies present
HIV-1 (–) HIV-2 (+)
Indicates HIV-2 antibodies present
HIV-1 (+) HIV-2 (+)
Indicates both HIV-1 and HIV-2 antibodies present
HIV-1 (–) HIV-2 (–) Or indeterminate
Indicates both HIV-1 and HIV-2 antibodies present
HIV-1 NAT
HIV-1 NAT (+)
Acute HIV-1 infection
HIV-1 NAT (–)
Negative for HIV-1
(+) = reactive test result; (–) = nonreactive rest result; NAT = nucleic acid test

FIGURE 18-8. CDC recommended HIV laboratory testing algorithm for serum/plasma samples.[73]

a fourth-generation test is reported as reactive, a supplemental assay that differentiates between HIV-1 and HIV-2 antibodies must be performed. If the supplemental antibody differentiation test result is nonreactive or indeterminate, an HIV-1 nucleic acid test (NAT) must be performed to confirm the diagnosis of HIV-1 infection.[73] As of this writing, no NAT has been approved for HIV-2 by the FDA, and CDC recommends consultation with expert in suspected cases of HIV-2 infection.

The Western blot (WB) was the confirmatory test of choice for detecting HIV-specific antibody for many years.[71,73,79,80] The WB is a protein electrophoretic immunoblot technique that detects specific antibodies to HIV protein and glycoprotein antigens. The proteins and glycoproteins are detected by WB as "bands" and can be divided into Env (envelope) glycoproteins (gp41, gp120, gp160), Gag or nuclear proteins (p17, p24/25, p55), and Pol or endonuclease-polymerase proteins (p34, p40, p52, p68). Criteria for the interpretation of WB results have been published by different health organizations and may vary depending on the issuing body. The WB traditionally was considered to have a low level of sensitivity and high specificity, but newer data showed the WB misinterpreted the majority of HIV-2 infections as being HIV-1.[73] The WB test is technically difficult to perform, expensive, associated with a relatively high rate of indeterminate results (4–20%) and has a long turnaround time (results are available in one to two weeks).[71,73,74] Because of these limitations and the availability of more reliable assays, the WB is no longer recommended for the diagnosis of HIV in the United States.[73]

Compared to the WB, the use of an HIV-1/HIV-2 differentiation assay detects HIV-1 antibodies earlier, reduces indeterminate results, identifies HIV-2 infections, has a shorter turnaround for test results and an overall lower cost.[73] To date, only the Multispot HIV-1/HIV-2 Rapid Test (Bio-Rad Laboratories, Redmond, WA) differentiation ELISA has been approved by the FDA as a supplemental test following a reactive fourth-generation HIV-1 and HIV-2 antibody and HIV-1 p24 antigen initial assay.[73,82]

Rapid HIV screening tests. Recent technological advances have allowed for the development of rapid (e.g., 30 minutes) screening tests for the detection of HIV antibodies. The methodology of the rapid HIV antibody assays involves typically either membrane EIA or immunocytochemical assays (ICA). Currently, nine FDA-approved rapid HIV-1 screening tests are available for use in the United States: OraQuick ADVANCE Rapid HIV-1/2 Antibody Test (OraSure Technologies, Bethlehem, PA); Uni-Gold Recombigen HIV-1/2 (Trinity Biotech, Jamestown, NY); Reveal Rapid HIV-1 Antibody Test (MedMira, Halifax, Nova Scotia); Multispot HIV-1/HIV-2 Rapid Test (Bio-Rad Laboratories, Redmond, WA); INSTI HIV-1/HIV-2 Antibody Test (bioLytical Laboratories, Richmond, BC); Alere Determine HIV-1/2 Ag/Ab Combo (Alere Scarborough, Scarborough, ME); (Clearview Complete) SURE CHECK HIV 1/2 Assay, (Clearview) HIV 1/2 STAT-PAK Assay and Chembio DPP HIV 1/2 Assay (Chembio Diagnostic Systems, Medford, NY).[71,78] The study and development of new rapid HIV tests continues, while an older testing method, Abbott's Single Use Diagnostic System HIV-1, has been removed from the market. All nine currently approved tests display specificity and sensitivity similar to ELISA, require only basic training in test performance and interpretation, can be stored at room temperature, and require minimal specialized laboratory equipment.[71,78] All assays, except for the Reveal Rapid HIV-1 Antibody Test and Multispot HIV-1/HIV-2 Rapid Test, have received waivers under the Clinical Laboratory Improvement Amendments, which allow point-of-care testing where dedicated laboratories are not available. Point-of-care tests are less complex to perform than traditional diagnostic tests and have minimal chance for error.[71,78,81]

Rapid HIV testing has proven useful for the detection of HIV infection in a number of clinical settings such as during the labor and delivery of pregnant women at high-risk of HIV infection with unknown serostatus for the purposes of preventing perinatal transmission, facilities where return rates for HIV test results are low, and following occupational exposure to potentially HIV-infected body fluids (e.g., through a needle stick) where immediate decisions regarding postexposure prophylaxis are needed.[71,78,80,83] It is important to note that rapid HIV tests are not recommended by the CDC for the diagnosis of HIV infection due to insufficient data, and a positive result from a rapid HIV test must be confirmed with a fourth-generation ELISA before the final diagnosis of HIV infection can be established. If the results of a rapid HIV test are negative, a person is considered uninfected. However, retesting should be considered in persons with possible exposure to HIV within the previous three months because the testing may have been performed too early in the infection to detect antibodies to the virus.[71,75,78]

Noninvasive HIV-1 tests. Several tests have been developed for testing HIV antibodies from oral fluid or urine.[71,73,80,83] The OraSure Western Blot Kit (OraSure Technologies, Bethlehem, PA) is a supplemental confirmatory test for the OraSure HIV-1 Oral Specimen Collection Device, which uses a cotton fiber pad that is placed between the cheek and lower gum for two minutes to collect oral mucosal transudate containing IgG. The OraSure HIV-1 oral device is FDA-approved and has a specificity of 99.4% (similar to EIA). The OraQuick ADVANCE Rapid HIV-1/2 Antibody Test (OraSure Technologies, Bethlehem, PA) detects antibodies against HIV-1 and HIV-2 by swabbing a flat pad against the upper and lower outer gums once, and inserting the sample collection pad into the developer solution vial. After 20 minutes, the results window will indicate whether HIV antibodies have been detected. Two urine-based HIV-1 tests (ELISA and WB) have also been FDA-approved for use but are associated with lower sensitivity and specificity than the oral fluid testing. As with ELISA testing of blood samples, confirmatory testing is required for both types of tests if the initial results are positive. Noninvasive HIV-1 testing should be considered in persons unable to access healthcare facilities, have poor venous access, or are reluctant to have their blood drawn. The advantages of these tests include avoidance of blood drawing for sample collection, ease of use, low cost, and stability of samples for up to 3 weeks at room temperature.[71,73]

Home sample collection tests. Several home sample collection tests for the detection of HIV infection are available over-the-counter.[73,80,83] For example, in the Home Access HIV-1 Test System (Home Access Health Corp., Hoffman Estates, IL), a few drops of whole blood are obtained using a finger stick and placed on a blood specimen card. The dried blood spot is mailed to a designated laboratory that uses ELISA to detect HIV-1 antibodies. The person calls the designated laboratory for the results (positive or negative for HIV antibodies) over the next three to seven business days. A telephone counselor provides the test results, telephone support, information, guidance about repeat testing, and referral as needed. An oral fluid home test kit is available. The OraQuick In-Home HIV Test (OraSure Technologies, Bethlehem, PA) requires swabbing upper and lower gums with a collection stick that is inserted into a test solution for 20 minutes, after which time the results can be read. A line on the control and test areas indicate a positive result. The advantages of home sample collection tests include ready access to HIV-1 testing, convenience, lower costs, anonymity, and privacy.[73]

p24 Antigen Tests

A main structural core protein of HIV is *p24,* with levels of p24 antigen being elevated during the early stages of HIV infection. Testing for p24 antigen has diagnostic utility during early infection when low levels of HIV antibody are present.[71,74] The direct detection of HIV-1 p24 antigen can be performed using a plasma or serum EIA assay.[73,80] Similar to antibody testing, positive results of the initial testing for HIV-1 p24 antigen must be retested in duplicate using the same EIA method. In addition, these results need to be confirmed with a neutralization assay due to the potential for p24 antigen testing to produce false-positive reactions resulting from interfering substances.[71,73]

The p24 antigen test may be used for screening blood donors, detecting growth in viral cultures, and as an alternative diagnostic test for HIV-1 in patients suspected of having acute HIV infection or infants <18 months of age born to HIV-infected mothers (Table 18-18).[71,73,80] The advantages of the p24 antigen test include the earlier detection of HIV infection compared to antibody testing (16 days versus 22 days); it is easier to perform; has a low cost; and has a specificity that approaches 100%. However, the DNA PCR assays and plasma HIV-RNA concentrations demonstrate greater sensitivity and have replaced the p24 antigen tests in many clinical situations.

HIV DNA PCR

HIV DNA PCR is used for early detection of proviral HIV-1 DNA in a patient's peripheral blood mononuclear cells. HIV DNA PCR is currently recommended for the diagnosis of HIV infection in infants (<18 months of age) born to HIV-infected mothers, and any clinical situations where antibody tests are inconclusive or undetectable (Table 18-18).[71,75] Antibody tests are not useful for diagnosing HIV infection in infants because maternal HIV antibodies can persist in the infant for up to 18 months after birth. Therefore, infants should be tested with the HIV DNA PCR or HIV RNA viral load test initially between 14 and 21 days of age, then at one to two months, and age three to six months.[75] Negative tests at birth can be repeated at 14 days of life because the assay sensitivity is increased by two weeks of life. To confirm the diagnosis of HIV infection, a positive result at any sampling time needs to be confirmed by a second HIV virologic test. HIV infection may be excluded in infants with two or more negative HIV virologic results when initial testing occurred at age ≥1 month and the second testing occurred at age ≥4 months.[75]

The advantages of the HIV DNA PCR test include a high level of sensitivity and specificity (96% and 99% at ~1 month of age, respectively), the requirement for only a small volume of blood (e.g., 200 μL), and the rapid turnaround time. The disadvantages include the expense, the high level of interlaboratory variability, and the availability of only one commercial assay (Roche Amplicor HIV DNA assay; Roche Diagnostics, Indianapolis, IN) that is not currently FDA-approved.[71,75]

HIV-RNA Concentration (HIV RNA Viral Load)

The accurate measurement of plasma *HIV-RNA concentrations* (also known as the *HIV viral load*) in conjunction with CD4$^+$ T lymphocyte count has become an essential component in the management of patients with HIV-1 infection.[71,75,76] These two laboratory tests provide the clinician with information regarding a patient's virologic and immunologic status, which is needed to make decisions regarding the initiation or changing of antiretroviral therapy and to predict the risk of disease progression from HIV infection to AIDS. In addition, plasma HIV-RNA concentrations can assist in the diagnosis of HIV infection in selected clinical situations (Table 18-18).[75,76]

Current methods that measure the amount of HIV-RNA in plasma include coupling reverse transcription to a DNA polymerase chain reaction (RT-PCR), identification of HIV-RNA with signal amplification by bDNA and NA sequence-based amplification (NASBA). Currently, there are five commercial assays approved by the FDA for clinical use: Amplicor HIV-1 Monitor version 1.5 (Roche Molecular Systems, Pleasanton, CA) in standard and ultrasensitive versions; Versant HIV-1 RNA 3.0 Assay (bDNA) (Siemens Healthcare Diagnostics, Tarrytown, NY); Cobas AmpliPrep/Cobas TaqMan HIV-1 versions 1 and 2 (Roche Diagnostics, Indianapolis, IN); and Real-Time TaqMan HIV-1 (Abbott Molecular, Des Plaines, IL).[71,76] The results of these tests are expressed as the number of HIV copies/mL. Higher HIV-RNA levels (e.g., >100,000 copies/mL) represent a substantial risk for disease progression. These assays differ in their dynamic ranges and lower limits of detection of HIV viral copies/mL of plasma. For example, the Versant assay has a lower limit of detection of <75 copies/mL while the Cobas AmpliPrep/Cobas TaqMan version 2 assay detection limit is <20 copies/mL. Some of these assays have different versions according to the degree of automation and simplicity. For instance, the Amplicor HIV-1 Monitor version 1.5 is a manual test; the Cobas Amplicor HIV-1 Monitor is semi-automated, and the Cobas AmpliPrep/Cobas Amplicor HIV-1 Monitor is automated. Additionally, the Amplicor HIV-1 Monitor and its variants exists as two FDA-approved assays; standard and ultrasensitive, due to its limited dynamic range. The standard assay has a lower limit of detection of 400 copies/mL compared to the ultrasensitive assay, which has a limit of 50 copies/mL. It is recommended to utilize both assays concurrently when testing samples that fall outside of the dynamic range. Therefore, if a viral load is reported as "undetectable," it signifies that the plasma HIV-RNA concentrations are below the lower limits of detection of the assay utilized.[71,76] When performing plasma HIV-RNA viral load levels for each patient, the same laboratory and method should be utilized to minimize variation.

Once the diagnosis of HIV-1 infection has been confirmed, a plasma HIV-RNA level should be measured to assist in the decision to start or defer therapy. Ideally, a plasma HIV-RNA level (and CD4$^+$ T lymphocyte count) is measured on two separate occasions as the baseline measurement. The decision to start antiretroviral therapy will depend on the clinical findings and symptoms of the patient, the results of the plasma HIV-RNA levels and CD4$^+$ T lymphocyte count, the willingness of the patient to adhere to therapy, and the potential risks associated with therapy. When initiating antiretroviral therapy, current guidelines recommend monitoring plasma HIV-RNA levels at the following time intervals: immediately before treatment initiation and two to eight weeks after starting or changing antiretroviral drug therapy; three to four months following therapy initiation and every three to four months while on therapy; and any time when clinically indicated, including those patients who experience a significant decline in CD4$^+$ T lymphocyte count. HIV-RNA testing is not recommended during the period of an acute illness (e.g., bacterial or *P. jirovecii* pneumonia) or in patients who have been recently vaccinated as these circumstances may increase the viral load for two to four weeks. For patients receiving antiretroviral therapy, the goals of therapy include specific reductions in the HIV viral load measured in log reductions over a given time frame as well as achieving a viral load "below the limits of detection." Changes in the amount of plasma HIV-RNA are often reported in log base 10 values. For example, a change from 10,000 to 1000 copies/mL in a patient on antiretroviral therapy would be considered a 1-log decrease in viral load. With optimal therapy, plasma HIV-RNA levels should decrease by ≥1 $\log_{10}$ during the first two to eight weeks after initiation of therapy and should continue to decline over subsequent weeks, with the ultimate goal of achieving an undetectable viral load (e.g., <50 copies/mL) in 16–24 weeks after initiation of therapy.[76] However, every patient responds differently. For patients who are not on therapy, plasma HIV-RNA levels should be monitored every three to four months to assess the patient's risk of disease progression and to determine the need for antiretroviral therapy. The reader is encouraged to review the most recent HIV diagnostic and treatment guidelines because recommendations for different patient populations are continuously being modified as newer data become available.[75-77]

CD4$^+$ T Lymphocyte Count

The cell-mediated immune function effects of HIV infection are demonstrated by reductions in the *CD4$^+$ T lymphocyte count*. Flow cytometry can be used to identify the various subsets of lymphocytes by their cluster of differentiation (CD) of specific monoclonal antibodies to surface antigens. The CD4$^+$ T lymphocytes are the helper-inducer T cells, whereas the

$CD8^+$ T lymphocytes are the cytotoxic-suppressor T cells. HIV infection causes a decrease in the total number of lymphocytes (particularly the $CD4^+$ T lymphocytes) as well as changes in the ratios of the different types of lymphocytes. $CD4^+$ T lymphocyte counts of <200 cells/mm^3 (normal count 800–1100 cells/mm^3) or a $CD4^+$ T lymphocyte percentage of <14% of the total lymphocyte count (normal 40% of total lymphocytes) are indicative of severe immunosuppression, placing the patient at risk for the development of opportunistic infections.[76]

As stated earlier, the $CD4^+$ T lymphocyte count is utilized in conjunction with the plasma HIV-RNA level to provide essential information regarding a patient's virologic and immunologic status, risk of disease progression from HIV infection to AIDS, and whether to initiate or change antiretroviral therapy.[75-77] Because of the significant impact on disease progression and survival, most guidelines recommend monitoring $CD4^+$ T lymphocyte counts at baseline and every three to six months to aid in the decision to initiate antiretroviral therapy, assess the immunologic response to treatment, and determine the need for any opportunistic infection chemoprophylaxis.[76] Current HIV treatment guidelines recommend initiation of antiretroviral therapy in all HIV-infected patients with a $CD4^+$ T lymphocyte count <350 cells/mm^3 regardless of the plasma HIV RNA viral load. Also, treatment should be offered to patients with $CD4^+$ T lymphocyte counts between 350 and 500 cells/mm^3. When the $CD4^+$ T lymphocyte count is >500 cells/mm^3, the decision to start therapy in asymptomatic patients is less clear and should be individualized. Therapy may need to be considered in patients with a high rate of decline in $CD4^+$ T lymphocyte (defined as >100 cell/mm^3 per year), even when the current count is 500 cells/mm^3 or greater. Additionally, antiretroviral therapy is recommended in patients with HIV-associated nephropathy, HBV co-infection when treatment for HBV is indicated, and patients who are pregnant regardless of $CD4^+$ T lymphocyte count. However, because HIV treatment recommendations are constantly evolving based on new data, the reader is referred to the most recent HIV treatment guidelines (https://aidsinfo.nih.gov/guidelines) for specific details on when antiretroviral therapy should be initiated in an individual patient based on clinical and laboratory parameters.

Phenotypic and Genotypic Assays for Antiretroviral Drug Resistance

Resistance of HIV-1 to antiretroviral drugs is an important cause for treatment failure. *Genotypic assays* utilize gene sequencing or probes to detect resistance mutations known to confer drug resistance in the RT, protease (PR), envelope, and integrase genes of HIV-1. Two genotypic assays have been approved by the FDA: TruGene (Siemens Healthcare Diagnostics, Tarrytown, NY) and ViroSeq (Abbott Molecular, DesPlaines, IL).[72] *Phenotypic assays* measure the quantity of viral replication in the presence of various concentrations of antiretroviral agents. Sequences from the RT, PR, envelope and integrase genes of the patient's HIV virus are inserted into a wild-type virus in the laboratory. The concentration of the drug needed to inhibit 50% of viral replication (IC_{50}) is reported. The ratio of IC values for the test and reference viruses is calculated and used to report the quantitative fold-increase in resistance of each antiretroviral agent. The interpretation of results from both assays is complex and requires expert knowledge and consultation.[73,76]

The current guidelines recommend drug resistance testing for patients with acute and chronic HIV infection when they enter into care, regardless of the decision to initiate antiretroviral therapy; pregnant women prior to antiretroviral therapy initiation; women contemplating or entering pregnancy with a detectable HIV-RNA level during treatment; patients with virologic failure while receiving antiretroviral therapy; and patients with suboptimal suppression of plasma HIV-RNA level after the initiation of antiretroviral therapy.[76]

Genotypic assays are typically recommended for resistance testing in treatment naïve patients and pregnant women. In the setting of virologic failure, current guidelines recommend performing resistance testing immediately after or within four weeks of the discontinuation of antiretroviral therapy for optimal results. Resistance testing is not recommended in patients with plasma HIV-RNA levels <1000 copies/mL.

The advantages and disadvantages of genotypic and phenotypic assays for the detection of HIV-1 resistance have been previously described.[73] The genotypic assay may be preferred over the phenotypic assay because of availability, clinical utility, faster turnaround time (one to two weeks versus two to three weeks), and lower cost.[72] However, both assays are complex, technically demanding, and expensive and are not routinely performed in most clinical laboratories.

Additional Laboratory Testing for HIV Patients

Coreceptor tropism assays. Following attachment of HIV to the $CD4^+$ T lymphocytes, fusion of the virus and $CD4^+$ cell membranes involves binding to a coreceptor molecule. The two coreceptors utilized by HIV are chemokine coreceptor 5 (CCR5) and CXC coreceptor (CXCR4). The recently approved antiretroviral agent, maraviroc, is a CCR5-coreceptor antagonist that prevents the entry of HIV into the $CD4^+$ cell by binding to the CCR5 receptor. Most acutely or recently infected patients harbor the CCR5-tropic virus, while untreated patients with advanced disease and those with disease progression shift from CCR5-tropic to CXCR4-tropic or both (dual-tropic or mixed-tropic). Treatment-experienced patients with high levels of drug resistance are more likely to harbor dual-tropic or mixed-tropic virus. Current HIV treatment guidelines recommend performing a coreceptor tropism assay when considering the use of a CCR5-coreceptor antagonist, or in the event of virologic failure during maraviroc therapy.[76] Currently, two phenotypic assays measure viral tropism; Phenoscript assay (VIRalliance, Paris, France) and Trofile assay (Monogram Biosciences, South San Francisco, CA), and one genotypic tropism assay, Genotypic Coreceptor Tropism Test, is available in the United States (Quest Diagnostics, Madison NJ), although it has a lower predictive value compared to the traditional phenotypic assay.[84]

HLA-B*5701 screening. Abacavir, a nucleoside reverse transcriptase inhibitor, is associated with a potentially life-threatening hypersensitivity reaction reported in 5–8% of patients in clinical trials. The hypersensitivity reaction appears to occur more frequently in white patients (5–8%) than black patients

(2–3%), and is associated with the presence of MHC class I allele HLA-B*5701. HIV treatment guidelines recommend screening patients for the presence of HLA-B*5701 prior to the initiation of abacavir-containing regimens in areas where the screening test is available, and those patients with a positive result should not receive abacavir. However, the HLA-B*5701 screening test may not be available in many clinical laboratories, therefore; the initiation of abacavir therapy can be reasonably considered using clinical judgment with appropriate monitoring and extensive patient education about the signs and symptoms of the hypersensitivity reaction.[76]

MYCOBACTERIA

Mycobacteria are nonmotile, nonspore-forming, aerobic bacilli that continue to cause infection as well as significant morbidity and mortality, especially in developing countries.[85-92] Currently, over 100 species of mycobacteria have been identified, with only a number of species causing infection in humans including *M. tuberculosis, M. leprae, M. avium complex, M. kansasii, M. fortuitum, M. chelonae, M. abscessus, and M. marinum.*[85–88] Depending on the species, mycobacteria can be nonpathogenic, pathogenic, or opportunistic and, therefore, may cause infection in both normal and immunocompromised hosts. **Table 18-19** lists the most common pathogenic mycobacteria species with their typical associated infections and environmental sources.[85-91]

Mycobacteria are generally divided into two groups based on epidemiology and spectrum of disease: (1) the *M. tuberculosis* complex including the species *M. tuberculosis*, *M. bovis, M. bovis* bacille Calmette-Guerin (BCG), *M. africanum*, and *M. microti*; and (2) nontuberculous mycobacteria (NTM; also referred to as *mycobacteria other than tuberculosis*), which include all other species of mycobacteria.[87,88] *M. tuberculosis* is the most clinically significant mycobacteria and is the causative organism of TB. The incidence of TB in the United States had been declining between 1953 (when it became a notifiable disease) and 1985 as a result of improved diagnostic methods, enhanced public health efforts to isolate patients infected with TB, and the introduction of effective antimycobacterial agents.[86,88,93] This decline in TB cases led experts to predict the elimination of the disease by 2010. However, between 1986 and 1992, an increase in the incidence of TB was observed in the United States due to deterioration of the TB public health programs, the emergence of the HIV epidemic, the increase in immigration to the United States, and the emergence of MDR-TB.[86,93] Because 1992, the number of cases of TB in the United States has steadily declined due to improved public health control strategies.[82] Despite the advances in medical care and treatment, TB continues to be one of the most common infectious diseases worldwide. The World Health Organization estimated that over 12 million persons were infected with TB worldwide in 2010, including over 8 million new cases and 1.6 million deaths that year.[94] Because TB can be transmitted from person-to-person, rapid diagnosis is necessary to decrease the spread of infection.[93]

The Identification of Mycobacteria

Mycobacteria possess a number of unique characteristics that contribute to the difficulties with the growth, identification, and treatment of these organisms. The cell wall of mycobacteria is complex and composed of peptidoglycan, polypeptides, and a lipid-rich hydrophobic layer.[87,88] This cell wall structure confers a number of distinguishing properties in the mycobacteria including (1) resistance to disinfectants and detergents; (2) the inability to be stained by many common laboratory identification stains; (3) the inability of mycobacteria to be decolorized by acid solutions (a characteristic that has given them the

TABLE 18-19. Pathogenic Mycobacteria and Associated Infections[85–91]

MYCOBACTERIUM SPECIES	ASSOCIATED INFECTIONS	ENVIRONMENTAL SOURCES
Mycobacterium tuberculosis complex (tuberculosis [TB])	Pulmonary infection, lymphadenitis, musculoskeletal infection, gastrointestinal infection, peritonitis, hepatitis, pericarditis	Humans
Mycobacterium bovis	Soft tissue infection, gastrointestinal infection	Humans, cattle
Mycobacterium leprae (Leprosy, Hansen disease)	Skin and soft tissue infections	Humans, armadillos
Mycobacterium avium complex (MAC)	Pulmonary infection, cutaneous ulcers, lymphadenitis, disseminated infection	Soil, water, swine, cattle, birds
Mycobacterium kansasii	Pulmonary infection, musculoskeletal infection, disseminated infection, cervical lymphadenitis	Water, cattle
Mycobacterium fortuitum	Skin and soft tissue infections, disseminated infection	Soil, water, animals, marine life
Mycobacterium chelonae	Skin and soft tissue infections, osteomyelitis, disseminated infection	Soil, water, animals, marine life
Mycobacterium abscessus	Skin and soft tissue infections, osteomyelitis, disseminated infection	Soil, water, animals, marine life
Mycobacterium marinum	Skin and soft tissue infections, bacteremia	Fish, fresh water, salt water
Mycobacterium ulcerans	Skin and soft tissue infections, osteomyelitis	Soil, stagnant water

name of acid-fast bacilli [AFB]); (4) the ability of mycobacteria to grow slowly; and (5) resistance to common anti-infective agents.[85,87,88] These characteristics have led to the continuous modification and improvement of laboratory practices used in the identification and diagnosis of mycobacterial infections. Many of these laboratory practices involve specialized staining techniques, growth media, identification techniques, environmental conditions (BSL-3 facilities for *M. tuberculosis*), and susceptibility testing methods that may be unavailable in some clinical laboratories.[88,90]

The ability to accurately cultivate mycobacteria is highly dependent on the appropriate selection and collection of biologic specimens for staining and culture.[3,88] Because different mycobacteria are capable of causing a number of infections (as listed in Table 18-19), the following biologic specimens may be submitted for mycobacterial culture based on the site of infection: respiratory tract secretions (e.g., expectorated or induced-sputum and bronchial washings) or gastric lavage specimens for the diagnosis of pulmonary TB; CSF for the diagnosis of TB meningitis; blood for the diagnosis of disseminated *Mycobacterium avium* complex (MAC) infection; stool for the diagnosis of disseminated MAC infection; and urine, tissue, exudate or wound drainage, bone marrow, sterile body fluids, lymph node tissue, and skin specimens for infection due to any mycobacteria.[86-90] For the diagnosis of pulmonary TB, several early morning expectorated or induced-sputum specimens are recommended to enhance diagnostic accuracy. Biologic specimens for mycobacterial culture should be immediately processed according to specified guidelines to prevent the overgrowth of bacteria that may also be present in the specimen, and should be concentrated to enhance diagnostic capability.[86,88,90]

Similar to the processing of specimens for bacterial culture, biologic specimens submitted for mycobacterial culture should be stained for microscopic examination and plated for culture. The staining techniques and culture media, however, are somewhat different because mycobacteria are poorly visualized in the Gram stain (they do not reliably take up the dyes and are referred to as *acid-fast*) and take longer to grow than conventional bacteria.

Staining with subsequent microscopic examination for mycobacteria is a rapid diagnostic test that involves the use of stains that are taken up by the lipid and mycolic acid components in the mycobacterial cell wall.[87,88] Several acid-fast stains are available for the microscopic examination of mycobacteria, including carbolfuchsin-based stains that are viewed using light microscopy (Ziehl-Neelsen or Kinyoun method) and fluorochrome stains (auramine-rhodamine) examined under fluorescence microscopy that are thought to be more sensitive tests, especially on direct specimens.[3,87-89,95] The sensitivity of the staining method is highly dependent on the type of clinical specimen, the species of mycobacteria present, the technique utilized in specimen processing, the thickness of the smear, and the experience of the laboratory technologist.[3,86-88] The staining methods can detect the presence of mycobacteria in a clinical specimen but cannot differentiate between species of mycobacteria. Therefore, several molecular techniques have been commercially developed to augment identification, which utilize NA amplification (PCR) to detect *M. tuberculosis* complex in acid-fast smear positive respiratory specimens (Amplicor *Mycobacterium tuberculosis* Test by Roche Diagnostics, Indianapolis, IN, and the Amplified *Mycobacterium tuberculosis* Direct Test by Gen-Probe) or acid-fast smear negative respiratory samples (Amplified *Mycobacterium tuberculosis* Direct Test by Gen-Probe).[3,85-88,90,92,95] Both of these tests display high sensitivity in detecting the presence of *M. tuberculosis* complex in smear-positive respiratory specimens (>97%).[3,88,95] Because of their high specificity and the rapid availability of results, the CDC recommends performing NA amplification tests on respiratory specimens of patients suspected of having pulmonary TB.[96,97] However, none of these assays are currently FDA-approved for the detection of *Mycobacterium* from non-respiratory specimens.[96,97]

For optimal cultivation and identification of mycobacteria, a combination of culture media, including at least one solid growth medium and one liquid growth medium, should be utilized during specimen processing to facilitate growth and optimize pigment production of the organism.[3,87-89,91,93,95] The preferred commercially available solid growth media for the cultivation of mycobacteria include an agar-based medium such as Middlebrook 7H10, or an egg-based medium such as Lowenstein-Jensen. There are several liquid growth media systems that are available for culture of mycobacteria, some of which employ continuous automated monitoring systems for the detection of mycobacterial growth.[3,87-89,91,92] The liquid growth media systems often provide more rapid isolation of AFB compared to conventional solid media, with results within 10 days as compared to 17 days or longer using solid growth media.[86,88-90,95] The most commonly used semiautomated systems with liquid growth media include the MB Redox (Heipha Diagnostika Biotest, Heidelberg, Germany), BACTEC 460TB system (BD, Franklin Lakes, NJ), the Septi-Chek AFB System (BD, Franklin Lakes, NJ), and the Mycobacteria Growth Indicator Tube (BD, Franklin Lakes, NJ).[3,87,88,91,95] The most commonly used automated, continuous monitoring systems with liquid growth media include the ESP Culture System II (Trek Diagnostics, Westlake, OH), the BACTEC 9000 MB System (BD, Franklin Lakes, NJ), the MB BacT/Alert System (bioMérieux, Marcy-l'Étoile, France), and the BACTEC MGIT 960 (BD Biosciences, Sparks, MD).[3,87,88,91,95] Once growth is detected in the liquid media systems, an acid-fast stain is performed on the specimen to confirm the presence of mycobacteria, with subsequent subculture onto solid media.

The optimal growth conditions of mycobacteria depend on the species; therefore, the clinical laboratory should follow a standardized procedure outlining the process that should be used to enhance cultivation of the suspected *Mycobacterium* from the submitted clinical specimen based on the suspected site of infection. The optimal conditions for incubation of mycobacterial cultures are 28–37 °C in 5–10% CO_2 for six to eight weeks, depending on the organism.[3,88,90,91] Cultures for mycobacteria typically require prolonged incubation periods, sometimes up to eight weeks, because most of the more common pathogens grow rather slowly. Rapidly growing mycobacteria such as *M. fortuitum, M. chelonae,* and *M. abscessus* typically grow within seven days on solid media, while slow growing mycobacteria such as *M. tuberculosis* complex, *M. avium* complex, *M. kansasii,* and *M. marinum* require seven days to seven weeks for growth.[87,89,91] Therefore, culture tubes or plates are examined weekly during the incubation period for the presence of mycobacterial growth.

Colonies grown in culture are examined microscopically for characteristic colonial morphologic features, pigmentation, and growth rate; are subjected to biochemical tests; and are evaluated using rapid molecular detection methods, such as PCR methods mentioned earlier, DNA hybridization using DNA probes, and chromatographic methods, such as high-performance liquid chromatography or gas liquid chromatography, to detect mycobacterial lipids) for definitive identification.[3,88-92] The DNA probes can be utilized only with mycobacteria grown in culture (not directly on patient specimens); are highly sensitive and specific; and are commercially available for the rapid identification of *M. tuberculosis* complex, *M. gordonae, M. kansasii,* and *M. avium* complex.[3,88-91] The molecular methods have replaced the use of biochemical tests in many laboratories because they provide more accurate identification in a significantly shorter time frame, within 14–21 days of specimen receipt as compared to several weeks or months using traditional identification methods.[86,92]

Susceptibility Testing of Mycobacteria

The choice of antibiotic or antimycobacterial agent to utilize in the treatment of mycobacterial infection depends on the species of mycobacteria involved. It is important for the clinician to have an understanding of the typical susceptibility patterns of specific mycobacterial species, the current treatment guidelines outlining which and how many drugs to use, and the methodology available for drug susceptibility testing for the particular mycobacterial species being treated.

Standardized guidelines have been published for the susceptibility testing of *M. tuberculosis* complex.[98] Susceptibility testing is currently recommended on the initial isolate of all patients with *M. tuberculosis* complex infection, on isolates from patients who remain culture positive after three months of appropriate therapy, and on isolates from patients who are not clinically responding to therapy.[3,88,93,98] Susceptibility testing of *M. tuberculosis* complex is initially performed with primary antituberculous agents such as isoniazid (using two concentrations, 0.2 and 1 mcg/mL), rifampin, ethambutol, and pyrazinamide. However, if resistance to any of these first-line drugs is detected, susceptibility testing should be subsequently performed using second-line drugs including streptomycin, a higher concentration of ethambutol (10 mcg/mL), ethionamide, capreomycin, ciprofloxacin, ofloxacin or levofloxacin, kanamycin, p-aminosalicylic acid, and rifabutin.[88,90,98]

Susceptibility testing of *M. tuberculosis* complex can be performed directly using mycobacteria from a smear-positive specimen (direct method) or using mycobacteria isolated from culture (indirect method).[88,90] The direct method of mycobacterial susceptibility testing produces faster results but is less standardized, so that susceptibility testing is usually performed using isolates grown in culture.[88,90] Four conventional methods are utilized worldwide for determining the susceptibility of *M. tuberculosis* isolates to antituberculous agents including the absolute concentration method, the resistance ratio method, the agar proportion method, and the agar proportion method using liquid medium (commercial radiometric, nonradiometric, or broth systems including the BACTEC 460TB System [BD, Franklin Lakes, NJ]; BACTEC MGIT 960 [BD, Franklin Lakes, NJ]; ESP Culture System II [Trek Diagnostics, Westlake, OH]; and MB/BacT-Alert 3D [bioMérieux, Marcy-l'Étoile, France]). The agar proportion method and the commercial broth systems are the methods most commonly used for mycobacterial susceptibility testing in the United States.

The agar proportion method is a modified agar dilution test that evaluates the extent of growth of a standardized inoculum of *M. tuberculosis* in control and drug-containing agar medium. The organism is considered resistant if growth is >1% on the agar plate containing critical concentrations of the antituberculous drug.[98] The critical concentration for each drug represents the lowest concentration of the drug that inhibits 95% of "wild type" *M. tuberculosis* strains that have never been exposed to the drug.[88,98] Susceptibility results using the agar proportion method are typically available 21 days after the plates have been inoculated.

The commercial susceptibility testing systems utilize liquid growth medium where the growth of the organism is measured in the presence and absence of antituberculous drugs.[86,88-91,95,98] These systems provide rapid susceptibility results for the primary antituberculous agents, but cannot be used for susceptibility testing of second-line agents. Commercial susceptibility tests are recommended over agar proportion methods because the results are often available within five to seven days after inoculation and can help guide appropriate therapy without the unnecessary delay of the agar proportion method.[86,90,91,98]

There are a number of other susceptibility testing methods that are currently being evaluated for drug susceptibility testing of *M. tuberculosis*. Many antimycobacterial drugs are now available in Etest strips, including streptomycin, ethambutol, isoniazid, and ethionamide.[92,95,98] Several studies have evaluated their performance as compared to the commercial methods; however, further studies are needed to validate the use of the Etest as a suitable, alternative susceptibility testing method. In addition, newer molecular methods for drug susceptibility testing of *M. tuberculosis* are currently being evaluated that are easier to perform and produce more reliable results in a shorter period of time.[88-92] These methods include PCR amplification, DNA sequencing line probe assays, or reverse hybridization-based probe assays for the detection of specific drug-resistance mutations. Further study, however, is warranted before newer susceptibility testing methods can replace conventional methods.

Guidelines for susceptibility testing of NTM have recently been published by the CLSI, where testing is recommended only on the initial isolate for clinically significant isolates (blood, tissue, etc.) that display variability in susceptibility to antituberculous drugs or for organisms that may be associated with acquired resistance.[98] The guidelines contain protocols for the susceptibility testing of rapidly growing NTM (*M. fortuitum, M. chelonae,* and *M. abscessus*) and slow-growing NTM (*M. avium* complex, *M. kansasii,* and *M. marinum*), including the recommended methodology and drugs to be tested for each organism.[87,88,90,98] Standard broth microdilution should be utilized to evaluate the susceptibility of any clinically significant, rapidly growing NTM.[89,98] Drugs that may be considered for susceptibility testing include amikacin, cefoxitin, ciprofloxacin, clarithromycin, doxycycline, imipenem, sulfamethoxazole, trimethoprim–sulfamethoxazole, and tobramycin (for *M. chelonae* only).[89,98] Susceptibility testing of *M. avium* complex is recommended for clarithromycin only using a broth-based method (macrodilution or microdilution) for initial blood or tissue isolates in patients with disseminated infection; for clinically significant isolates from patients receiving previous or current macrolide therapy; for isolates from patients who develop bacteremia while receiving macrolide prophylaxis; and for isolates from patients who relapse while receiving macrolide therapy.[89,98] Regarding the other slow-growing NTM, susceptibility testing of *M. kansasii* should be routinely performed only on rifampin using the commercial radiometric systems, broth microdilution, or the modified proportion method; while routine susceptibility testing of *M. marinum* is not recommended.[89,98]

Skin Testing

The *Mantoux test* or *tuberculin skin test* (TST) is one test available for the detection of latent TB, and involves the intradermal injection of a purified protein derivative (PPD) of the tubercle bacilli, which is obtained from a culture filtrate derived by protein precipitation.[90,99] Injection of the PPD into individuals previously exposed to TB will elicit a delayed hypersensitivity reaction involving T cells that migrate to the area of intradermal injection (usually the dorsal aspect of the forearm), inducing the release of lymphokines that produce induration and edema within 48–72 hours after injection. The diameter of induration is measured between 48–72 hours after injection by a healthcare professional.[90,99] Published guidelines are available for interpretation of the TST reaction based on the size of the induration and clinical and demographic characteristics of the patient. An induration of ≥5 mm is considered positive in persons at high risk of developing TB disease including HIV infected patients; patients receiving immunosuppressive therapy; patients who have been recently exposed to a person with TB; and patients with an abnormal chest radiographic consistent with prior TB.[85,90,99] An induration of ≥10 mm is considered positive in patients who are not immunocompromised and possess no other identified risk factors for developing tuberculous disease such as recent immigrants from high prevalence countries; injection drug users; residents and employees of high-risk settings (e.g., prisons, healthcare facilities, and mycobacteria laboratory personnel); persons with chronic medical conditions of high risk (e.g., diabetes, silicosis, and chronic renal failure), and children younger than four years of age.[85,90,99] An induration of ≥15 mm is considered positive in persons at low risk for developing active infection with TB.[85,90,99]

A two-step TST is recommended by the CDC in certain populations (*initial* skin testing of newly hired healthcare workers without a documented negative TST within the past 12 months and persons expected to undergo serial screening for TB, such as residents and staff of long-term care facilities) to identify those individuals with past TB infection whose delayed-type hypersensitivity to tuberculin has diminished over time.[100,101] The first TST is administered as described above, with a second TST administered following the same procedure one to three weeks later in persons with a negative initial test.[100] The premise behind the administration of two TSTs in these settings is to delineate between past TB infection or BCG vaccination from recent conversion/infection. That is, the first injection will stimulate (boost) the delayed hypersensitivity response in a patient with previous TB infection or BCG vaccination and that the second TST will then elicit a positive reaction.[100,101]

Blood Assay for Mycobacterium Tuberculosis

Two in vitro diagnostic tests using whole blood have become recently available in the United States for the detection of latent *M. tuberculosis* infection: (1) a blood assay for *M. tuberculosis* (BAMT), the QuantiFERON-TB Gold In-Tube test (Cellestis, Chadstone, Victoria, Australia), and (2) T-SPOT.TB (Oxford Immunotec, Oxford, United Kingdom).[86,88] These tests utilize ELISA to measure the amount of interferon gamma (IFN-γ) released from sensitized lymphocytes from prior exposure to *M. tuberculosis* following overnight incubation with PPD from *M. tuberculosis* and control antigens.[100,102] Because the tests utilize peptide antigens from *M. tuberculosis,* they have more specificity than the TST for the diagnosis of latent *M. tuberculosis* infection and will not produce false-positive results in patients with previous BCG vaccination or infection due to NTM.[100,102] The results of BAMT testing are stratified according to risk for TB infection (like the TST) but are not influenced by the subjectivity of reader bias or error, as may be seen with the TST. Because these tests are unable to distinguish between active or latent infection, the exact role of the BAMT and the TST in the diagnosis of latent TB infection are unclear and currently under investigation. However, the BAMT test can be used to assist in the diagnosis of latent TB infection in high-risk populations such as recent immigrants from high-prevalence countries, injection drug users, inmates, prison employees, and healthcare workers at high risk for exposure to TB.[100,102] The BAMT test can also be considered as the initial and serial screening test for latent TB infection in healthcare workers and military personnel.[100,102] It is important to note that the FDA has approved these tests as aids in the diagnosis of *M. tuberculosis* infection and are intended to be used in conjunction with other diagnostic techiques.[102]

LABORATORY TESTS UTILIZED FOR THE IDENTIFICATION OF UNCOMMON OR MISCELLANEOUS ORGANISMS

There are a number of pathogenic organisms that are difficult to detect or cultivate using the standard microbiologic procedures outlined above. These organisms often pose a diagnostic dilemma because they often require specialized testing for identification. It is beyond the scope of this chapter to describe all of the specialized testing methods that are available to detect these organisms; however, an abbreviated list can be found in **Table 18-20**.[4,13,43,103-130]

LABORATORY TESTS UTILIZED FOR THE DIAGNOSIS OF SPECIFIC INFECTIONS

Meningitis

Meningitis is an infectious diseases medical emergency requiring prompt, accurate diagnosis and treatment. Meningitis may be caused by bacteria, viruses, fungi, or mycobacteria, and produces a resulting clinical presentation of acute or chronic meningitis depending on the causative organism. In a patient with suspected meningitis, a lumbar puncture is performed to obtain CSF for laboratory analysis to aid in the diagnosis of the infection, including the potential causative organism.[2,131-137] In patients who present with papilledema, altered consciousness, new onset seizures, or focal neurologic findings, a head computed tomography may be performed prior to the lumbar puncture to exclude the presence of a space-occupying lesion, which may put the patient at risk for brain herniation after lumbar puncture.[132] A lumbar puncture involves the aseptic insertion of a spinal needle into the subarachnoid space at the lumbar spine level for the aspiration of 5–20 mL of CSF for analysis.[131,133] When initiating the lumber puncture, the opening pressure may be measured (normal opening pressure = 50–195 mm H_20 in adults), and is often elevated in patients with meningitis (especially *C. neoformans* meningitis) and concomitant cerebral edema or intracranial focus of infection.[132,135] The CSF should be placed in three to four separate sterile screw-cap tubes and immediately transported to the laboratory for rapid processing. The first two tubes of CSF are processed for microbiologic (e.g., Gram stain, fungal stains, AFB stain, culture, and antigen detection) and chemical studies (e.g., general appearance, glucose, and protein), while the last two tubes are processed for determination of the WBC count with differential as described below.[2] The typical CSF

TABLE 18-20. Specialized Laboratory Tests for the Detection of Specific Organisms[4,13,43,103-130]

ORGANISM	TYPE OF ORGANISM	CLINICAL FINDINGS AND INFECTIONS	DIAGNOSTIC METHOD	POSITIVE RESULT	REFERENCES
Bordetella pertussis (Whooping cough)	Bacteria	Upper respiratory tract symptoms, characteristic whooping cough, pneumonia	Culture	Growth within 3–7 days; more sensitive when performed early in course of infection	103–105
			DFA, ELISA	Detection of *B. pertussis* antibodies; should be used in conjunction with culture due to variable specificity	
			PCR	Direct detection in 1–2 days; most sensitive when performed early in course of infection	
Borrelia burgdorferi (Lyme disease)	Spirochete	Erythema migrans, pericarditis, arthritis, neurologic disease	Culture	Not routinely available; long incubation (hold cultures for up to 12 wk)	13, 106, 107
			ELISA (or rarely IFA); first step screening test	Measures IgM (appears within 1–2 wk) and IgG (appears within 4–6 wk) antibodies against *B. burgdorferi*	
			WB if ELISA borderline or positive, second step confirmatory test	Measures IgM and IgG antibodies against *B. burgdorferi*	
			PCR (confirm)	Can detect low numbers of spirochetes; especially useful in synovial fluid	

(*continued*)

TABLE 18-20. Specialized Laboratory Tests for the Detection of Specific Organisms[4,13,43,103-130], cont'd

ORGANISM	TYPE OF ORGANISM	CLINICAL FINDINGS AND INFECTIONS	DIAGNOSTIC METHOD	POSITIVE RESULT	REFERENCES
Brucella spp.	Bacteria	Systemic infection (can involve any organ); spondylitis, arthritis, endocarditis	Culture	Growth within 7 days, but cultures should be held for 3 wk	108, 109
			SAT, MAT	Detects antibodies to most *Brucella* spp.; titer of ≥1:160 is diagnostic in conjunction with appropriate clinical scenario	
			ELISA, IFA	Useful for detection of chronic or past brucellosis; most useful for diagnosis of neurobrucellosis	
			PCR	Detection of *Brucella*-specific DNA sequences; not routinely available in most laboratories	
Chlamydia pneumoniae	Atypical bacteria	Upper respiratory tract infections, pharyngitis; pneumonia	Culture	Monoclonal antibodies detect organism in culture	4, 13, 103, 110, 111
			CF	Fourfold rise in antibody titer between paired sera (acute and convalescent samples)	
			MIF	Fourfold rise in antibody titer between paired sera (acute and convalescent samples) or a single serum sample with an IgM titer of 1:16 or an IgG titer 1:512	
			EIA	Detection of IgG, IgM, or IgA against *C. pneumoniae*	
			PCR	Detection of *C. pneumoniae* DNA	
Clostridium difficile (Pseudomembranous colitis)	Anaerobic bacteria	Pseudomembranous colitis, diarrhea	Culture using CCFA growth media	Growth within 48 hr; most sensitive test	43, 112, 113
			CCNA or EIA toxin test	Detection of toxin A or B activity; cell cytotoxicity test more sensitive than EIA	
			PCR	Rapid sensitive and specific detection of *C. difficile tcdB* gene	
Coxiella burnetti (Q fever)	Bacteria	Acute or chronic systemic illness, pneumonia, hepatitis, endocarditis	Culture with DFA	Growth in 6–14 days, organism detected by DFA	13, 103, 110
			IFA, EIA, CF	IgM titer of 1:50 or an IgG titer of 1:200	
Cryptosporidium parvum	Protozoa	Acute diarrhea (self-limiting to severe), abdominal pain, dehydration	Modified acid fast staining	Detection of oocysts in stool or intestinal scrapings	114, 115
			DFA using a monoclonal antibody against oocyst	Detection of oocysts in stool or intestinal scrapings	
			EIA	Detection of *C. parvum* antigen in stool or intestinal scrapings	
			PCR	Detection and differentiation of *Cryptosporidium* spp.	

TABLE 18-20. Specialized Laboratory Tests for the Detection of Specific Organisms[4,13,43,103-130], cont'd

ORGANISM	TYPE OF ORGANISM	CLINICAL FINDINGS AND INFECTIONS	DIAGNOSTIC METHOD	POSITIVE RESULT	REFERENCES
Ehrlichia spp.	Bacteria	HME and HGA—fever, myalgia, headache, malaise, leukopenia, thrombocytopenia, and elevated AST and ALT; may be life-threatening	Indirect immunofluorescence serology	Single sample IgG titer of >1:128 or a fourfold rise in antibody titer between paired sera (acute and convalescent samples)	110, 116, 117
			Peripheral blood smear Wright stain	Intracytoplasmic inclusions	
			PCR	Detection of *E. chaffeensis* or *E. phagocytophilum* DNA sequences	
Entamoeba histolytica	Protozoa	Amebiasis: intestinal (colitis, diarrhea) and extraintestinal (liver abscess)	Stool exam for ova and parasites (O&P)	Detection of trophozoites and cysts	114, 118, 119
			Culture using axenic or xenic methods	Growth of *E. histolytica*	
			Serology (indirect FA, LA, ID, CF, EIA)	Detection of *E. histolytica* antibodies	
			Antigen detection on fresh stool samples	Detection of *E. histolytica* or *E. dispar* specific antigen	
			PCR	Detection and differentiation of *E. histolytica* or *E. dispar*	
Giardia lamblia	Protozoa	Acute diarrhea (self-limiting to severe), malabsorption syndromes, low-grade fever, chills, abdominal pain	Stool exam for ova and parasites (O&P)	Detection of trophozoites and/or cysts	114
			Wet preps or stains of duodenal material	Detection of trophozoites and/or cysts	
			EIA, DFA, or ICA antigen detection assays	Detection of trophozoite and/or cyst antigens	
Helicobacter pylori	Bacteria	Peptic ulcer disease	Urea breath test	Positive test indicative of the presence of organism	120, 121
			Serologic tests	Detect IgG antibodies against *H. pylori*	
			Stool antigen assays (ELISA)	Detection of *H. pylori* antigen	
			Urease test on antral biopsy specimen	Positive test indicative of active infection	
			PNA-FISH, PCR	Detection of *H. pylori* DNA	
Legionella pneumophila	Atypical bacteria	Pneumonia	Culture (using specialized media)	Growth in 3–5 days	4, 103, 122, 123
			DFA staining	Binds to *L. pneumophila* antigen to produce fluorescence	
			IFA serology	Fourfold rise in antibody titer between paired sera (acute and convalescent samples)	
			Urinary antigen detection (EIA, RIA, ICA)	Detects *Legionella pneumophila* serogroup 1 antigen only	
			PCR	Detection of *Legionella* spp. DNA	

(continued)

TABLE 18-20. Specialized Laboratory Tests for the Detection of Specific Organisms[4,13,43,103-130], cont'd

ORGANISM	TYPE OF ORGANISM	CLINICAL FINDINGS AND INFECTIONS	DIAGNOSTIC METHOD	POSITIVE RESULT	REFERENCES
Leishmania spp.	Protozoa	Cutaneous, mucocutaneous, or visceral (VL, kala-azar) infection; can infect reticuloendothelial system	Giemsa staining and light microscopy	Amastigotes within the specimen	114, 124
			Culture	Growth of promastigotes	
			IFA, DAT, ELISA (VL)	Detection of anti-leishmanial antibodies in blood or serum	
			LA (VL)	Detection of leishmanial antigen in urine	
			PCR (VL)	Detection of *Leishmania* DNA	
Leptospira spp.	Spirochetes	Leptospirosis (self-limiting with fevers, chills, myalgia, headache, aseptic meningitis); icteric leptospirosis (severe form associated with jaundice, bleeding, and renal failure)	Dark-field microscopy or immunofluorescence	Detection of motile leptospires	13,125
			Culture	Growth within 6 wk	
			Serology using MAT	Fourfold or greater rise in agglutinating antibody titer between paired sera (acute and convalescent samples)	
			ELISA	Detection of leptospiral antibodies	
			PCR	Detection of leptospiral DNA	
Mycoplasma hominis	Atypical bacteria	Urogenital tract infections including prostatitis, PID, bacterial vaginosis, urethritis; systemic infection in neonates and immunocompromised patients	Culture using selective media	Growth within 5 days	13, 126, 127
Mycoplasma pneumoniae	Atypical bacteria	Pneumonia, tracheobronchitis, pharyngitis	EIA serology	Fourfold or greater rise in antibody titer between paired sera (acute and convalescent samples)	4, 13, 103, 126, 127
			NAAT, PCR	Detection of *M. pneumoniae* DNA	
Plasmodium falciparum, P. vivax, P. ovale, P. malariae (Malaria)	Protozoa	Symptoms include high fever (cyclic with *P. vivax, P. ovale, P. malariae*), chills, nausea, vomiting, severe headache, anemia, abdominal pain; life-threatening with *P. falciparum*	Thick and thin blood films stained with Giemsa stain (gold standard)	Presence of malarial parasites	114, 128
			Fluorescent-assisted microscopy	Detection of fluorescence when dyes are taken up by the nucleus of the parasite	
			PCR (only in well-equipped laboratories)	Detection of malaria-specific DNA sequences	
			ICA antigen assays	Detection of malaria-specific antigens	

TABLE 18-20. Specialized Laboratory Tests for the Detection of Specific Organisms[4,13,43,103-130], cont'd

ORGANISM	TYPE OF ORGANISM	CLINICAL FINDINGS AND INFECTIONS	DIAGNOSTIC METHOD	POSITIVE RESULT	REFERENCES
Pneumocystis carinii (*jirovecii*)	Fungus with protozoal characteristics	Pneumonia, extrapulmonary infection	Microscopic exam after stain of induced sputum, BAL specimen or tissue biopsy	Detection of trophic or cystic forms	129
			DFA or IFA	Detection of cysts or trophozoites	
			PCR	Detection of *P. jirovecii*-specific DNA	
Rickettsia rickettsii (Rocky Mountain spotted fever [RMSF])	Rickettsia	Fever, chills, headache and rash in patient with recent tick bite; myalgias, malaise, nausea, vomiting, abdominal pain, focal neurologic findings; small vessel vasculitis may result in life-threatening complications	IFA (gold standard)	Fourfold or greater rise in IgM or IgG antibody titers between paired sera (acute and convalescent samples)	110,117
			EIA or LA	Detection of IgM or IgG antibodies	
			PCR	Detection of *R. rickettsii* DNA sequences	
Strongyloides stercoralis	Parasite	Abdominal infection; disseminated infection (hyperinfection syndrome with pneumonitis, sepsis)	Stool exam for ova and parasites (O & P)	Detection of adult worms, eggs and/or larvae	114
Taenia solium	Tapeworm	Neurocysticercosis (infection within brain tissue) causing seizures, headache, focal neurologic deficits; muscular and subcutaneous abscesses	Serology by EITB	Detection of antibodies to *T. solium* glycoprotein antigens	114
			ELISA on CSF	Detection of anticysticercal antibodies or cysticercal antigens	
Toxoplasma gondii (Toxoplasmosis)	Protozoa	Encephalitis, myocarditis, lymphadenitis, polymyositis, chorioretinitis, toxoplasmosis during pregnancy, congenital toxoplasmosis	Serology testing by Sabin-Feldman Dye test, IFA, ELISA, IgG avidity test, agglutination	Positive IgG antibody	114, 130
			Giemsa or Diff-Quik stain of CSF or body fluid/tissue	Demonstration of tachyzoites	
			PCR	Detection of *T. gondii*-specific DNA	
Ureaplasma urealyticum	Atypical bacteria	Urogenital tract infections including prostatitis, PID, bacterial vaginosis, urethritis; systemic infection in neonates and immunocompromised	Culture using selective media	Growth within 5 days	13,126, 127
			PCR	Detection of NA or gene targets	

ALT = alanine aminotransferase; AST = aspartate aminotransferase; BAL = bronchoalveolar lavage; CCFA = cycloserine cefoxitin fructose agar; CCNA = cell cytotoxicity neutralization assay; CF = complement fixation; CSF = cerebrospinal fluid; DAT = direct agglutination test; DFA = direct fluorescent antibody; DNA = deoxyribonucleic acid; EIA = enzyme immunoassay; ELISA = enzyme-linked immunosorbent assay; EITB = enzyme-linked immunoelectrotransfer blot; HGA = human granulocytic anaplasmosis; HME = human monocytic ehrlichiosis; ICA = immunochromatographic assay; ID = immunodiffusion; IgG = Immunoglobulin G; IgM = immunoglobulin M; IFA = immunofluorescent assay/indirect fluorescent antibody; LA = latex agglutination; MAT = microagglutination test; MIF = microimmunofluorescence; NA = nucleic acid; NAAT = nucleic acid amplification test; PCR = polymerase chain reaction; PID = pelvic inflammatory disease; RIA = radioimmunoassay; PNA-FISH = peptide nucleic acid fluorescent in situ hybridization; SAT = serum agglutination test; VL = visceral leishmaniasis; WB = western blot.

chemistry, hematology, and microbiologic findings in patients with meningitis caused by different pathogens are listed in **Table 18-21.**[132-137]

Chemistry and Hematology

In patients with meningitis, the CSF often appears cloudy due to the presence of WBCs, protein, and bacteria.[132] The chemistry and hematology results from the CSF analysis directly correlate with the probability of infection, so that negative findings exclude the likelihood of meningitis in almost all cases.[132,133,135,136] Patients with acute bacterial meningitis often demonstrate marked abnormalities in the chemistry analysis of the CSF, with protein concentrations of >100 mg/dL and glucose concentrations <45 mg/dL (or a CSF:blood glucose ratio of <0.5) due, in part, to disruption of the blood brain barrier.[132,135,136]

Hematologic analysis of the CSF involves the determination of the WBC count with corresponding WBC differential, which may suggest the potential causative organism of the meningitis. Patients with acute bacterial meningitis often demonstrate an elevated CSF WBC count (>400 cells/mm^3) with a neutrophilic predominance (>80% neutrophils). In contrast, patients with viral, fungal, or mycobacterial meningitis often display lower CSF WBC counts (5–200 cells/mm^3) with a predominance of lymphocytes.

CSF Stain and Culture

For patients with suspected bacterial meningitis, a Gram stain and culture should be performed on CSF. The Gram stain will demonstrate an organism in 60–90% of patients with bacterial meningitis and is helpful in selecting appropriate empiric antibiotic therapy.[2,132,134-136] However, the sensitivity of the CSF Gram stain diminishes to 40–60% in patients who have received antibiotics prior to the lumber puncture (also known as *partially treated meningitis*).[2,134] In patients with meningitis due to viruses, fungi, or mycobacteria, the Gram stain is usually negative and specialized tests should be used, such as the India ink stain or cryptococcal antigen test for the detection of *C. neoformans* or the acid-fast stain for the detection of *M. tuberculosis.*

All CSF specimens should be processed for culture based on the type of meningitis (acute versus chronic) and the organism suspected of causing the infection. In patients with bacterial meningitis, the cultures are often positive within 24–48 hours. In patients with nonbacterial meningitis, culture specimens should be incubated for longer periods of time (up to two to six weeks), because these organisms often take longer to grow.

Other Specialized Tests

Several specialized tests may be performed on CSF specimens to aid in the detection of the causative organism. These tests include bacterial antigen detection using LA, latex fixation, or EIA; fungal antigen detection; antibody detection; and bacterial, viral, or mycobacterial PCR assay.[132-137]

Bacterial antigen testing on CSF specimens is considered a rapid diagnostic test because the results are available well before the results of the CSF culture. The commercially available tests utilize antibody-coated particles that bind to specific capsular antigens of the most common pathogens that cause acute bacterial meningitis, including *S. pneumoniae, N. meningitidis, H. influenzae* type B, and group B streptococci. The tests are performed by combining CSF (although it can also be performed using urine or serum) with antibody-coated particles and observing for agglutination, which signifies the presence of the bacterial antigen in the specimen. If visible agglutination does not occur, either the antigen is not present or it is present in insufficient amounts to cause detectable agglutination. Routine bacterial antigen detection of CSF specimens is not recommended because the tests are not more sensitive than the traditional Gram stain, the results lack high specificity or sensitivity, the results rarely impact patient treatment, and their use has not been shown to be cost-effective.[3,132,137] However, bacterial antigen testing may be useful in patients with negative Gram stains or in patients that have received previous antimicrobial therapy.[2,132-134,136,137]

Another antigen test has recently become available, the Alere Binax NOW *S. pneumoniae* antigen test, to aid in the diagnosis of meningitis due to *S. pneumoniae*.[138] It is a rapid ICA test that detects the presence of the C-polysaccharide antigen of the *S. pneumoniae* cell wall in the CSF of patients suspected of

TABLE 18-21. Typical CSF Findings in Patients with Meningitis[132-137]

	NORMAL	BACTERIAL MENINGITIS	VIRAL INFECTION	FUNGAL MENINGITIS	TUBERCULOUS MENINGITIS
Opening pressure (mm H_2O)	<180	>195			
WBCs (count/mm^3)	0–5	400–20,000 (mean 800)	5–2000 (mean 80)	20–2000 (mean 100)	5–2000 (mean 200)
WBC differential	No predominance	≥80% PMNs	>50% lymphs, 20% PMNs	>50% lymphs	>80% lymphs
Protein (mg/dL)	<50	>100	30–150	40–150	>50
Glucose (mg/dL)	45–100 (⅔ of serum)	<45 (<½ of serum)	45–70	30–70	<40
Gram stain (% positive)	—	60–90	Negative	Negative	37–87 (AFB smear)

AFB = acid-fast bacilli; CSF = cerebrospinal fluid; PMNs = polymorphonuclear leukocytes; lymphs = lymphocytes; WBCs = white blood cells.

having pneumococcal meningitis.[138] The test is highly sensitive and specific with results available within 15 minutes.[138] In addition, the test is not influenced by previous antibiotic therapy. The results of the antigen test are considered presumptive and should be utilized with the results of culture in when establishing the diagnosis of pneumococcal meningitis.

Fungal antigen testing on CSF is available for *H. capsulatum, B. dermatitidis*, and *C. neoformans*.[133,137] The antigen testing for *C. neoformans* has become an important diagnostic tool that has become useful in predicting the course of infection and monitoring the response to antifungal therapy. For some organisms, diagnostic tests that detect the presence of antibody in the CSF are available, including syphilis (*T. pallidum*), Lyme disease (*B. burgdorferi*), and *Coccidioides immitis*.[52,53,106,107,133,134]

PCR assays are the most useful tests for the diagnosis of viral infections in the CNS and can be used to detect the presence of HSV, CMV, VZV, EBV, and enterovirus in the CSF.[64,132,133] The PCR assay involves the amplification of small amounts of specific DNA of the target organism followed by subsequent identification and verification. PCR is also available for the detection of *S. pneumoniae, N. meningitidis, Listeria monocytogenes,* and *M. tuberculosis*; however, the time and expense of performing PCR assays for organisms other than viruses and *M. tuberculosis* has limited their routine clinical use.[132,133]

Streptococcal Pharyngitis

Acute pharyngitis is one of the most common infections encountered in medicine and can occur in both children and adults. Acute pharyngitis can be caused by a number of organisms (e.g., bacteria and viruses), which produce similar signs and symptoms of infection. Antibiotic therapy is recommended only for patients with pharyngitis due to bacteria, especially group A streptococci (*Streptococcus pyogenes*).[139] Because group A strep pharyngitis comprises only a small percentage (20–30%) of patients with acute pharyngitis, it is important that a rapid, reliable diagnostic test be available to avoid unnecessary antibiotic use in patients with acute viral pharyngitis.[139]

The gold standard diagnostic test for acute pharyngitis due to group A streptococcus is the throat culture, which often takes one to two days for results. Therefore, rapid antigen detection tests (RADTs) have been developed to expedite and confirm the diagnosis of group A streptococcal pharyngitis, with most tests yielding results within 15 minutes.[139,140] Positive RADT tests expedite the initiation of antibiotic treatment in the appropriate patient. There are several RADT tests that are commercially available, with the newer tests employing EIA or chemiluminescent DNA probes (>95% specificity and ≥90% sensitivity).[139,140] There are limited studies comparing the performance of different RADT tests to throat culture (the gold standard), so current recommendations suggest that traditional throat culture be performed in children and adolescents with a negative RADT test to definitively exclude group A streptococcal pharyngitis.[139,140]

Pneumonia

There are a number of obstacles that make the diagnosis of bacterial pneumonia quite difficult. First, the respiratory tract is colonized with bacteria that may or may not be contributing to the infectious process. When obtaining a sample for culture, lower respiratory tract secretions can become contaminated with secretions or bacteria colonizing the upper respiratory tract; therefore, expectorated sputum samples should be evaluated to determine if contamination with saliva or upper respiratory tract flora has occurred (assessment of the adequacy of the sample).[1,2,4,6,7,131,141,142] If bacteria other than normal respiratory flora are isolated, the clinician must determine the relative importance and significance of the organism(s) isolated as a potential cause of pneumonia by assessing the presence of signs and symptoms of respiratory tract infection in the patient. In some patients, adequate sputum specimens are difficult to obtain without invasive procedures such as BAL or protected specimen brush (PSB). Invasive procedures are occasionally utilized to aid in the diagnosis of pneumonia in patients who are not able to expectorate an adequate sputum sample, in immunocompromised patients, and in patients with HAP or ventilator-associated pneumonia (VAP).[2,19,141,143] Despite the best efforts at obtaining a lower respiratory tract sputum specimen for culture, as many as 30–50% of patients with pneumonia have negative cultures.[141,142]

To obtain an adequate expectorated sputum sample, the patient should be instructed to provide sputum generated from a deep cough. All expectorated sputum samples should be screened to ensure that the specimen is adequate and has not been contaminated by saliva or upper respiratory tract flora prior to processing for culture.[2,131,141,142] Information utilized to assess the adequacy of an expectorated sputum sample is derived from visualization of the Gram stain of the specimen. Expectorated sputum specimens that contain >25 WBCs/hpf (unless the patient is neutropenic) and <10 squamous epithelial cells/hpf are considered adequate for further processing and culture.[4,141,142] Samples with >10 epithelial cells/hpf are representative of upper respiratory tract contamination (saliva) and should not be processed for culture. The sputum Gram stain from an adequate sputum specimen may be used to guide empiric antibiotic therapy when the specimen is purulent and contains a predominant organism. Antibiotic therapy should be modified based on the culture results, especially if they reveal an infecting organism.

A noninvasive test, the Alere Binax NOW *S. pneumoniae* antigen test, is available to aid in the diagnosis of pneumonia due to *S. pneumoniae*.[2,3,138,141,143] It is a rapid ICA test that detects the C-polysaccharide antigen of the *S. pneumoniae* cell wall in the urine of patients suspected of having pneumococcal pneumonia (86% sensitivity and 94% specificity).[138,141,143] The results of the test are available within 15 minutes and are not influenced by previous antibiotic therapy.[138,141-143] The *S. pneumoniae* urinary antigen test should be considered in patients when a sputum sample for culture cannot be obtained in a timely fashion, in patients with severe pneumonia requiring ICU admission, in patients at risk for pneumococcal pneumonia (asplenic, alcohol abuse, liver disease), in patients with pneumonia and concomitant pleural effusion, in patients who failed previous outpatient antibiotic therapy, and in patients who have received antibiotics before a specimen for culture has been obtained.[142,143] Results of the urinary antigen test are presumptive and should be utilized with the results of the sputum culture when establishing the diagnosis of pneumococcal pneumonia.

In the case of pneumonia caused by atypical bacteria such as *L. pneumophila, Mycoplasma pneumoniae,* or *Chlamydophila pneumoniae,* antigen detection tests (*Legionella* urinary antigen test for the detection of *Legionella* serogroup 1), serologic tests, or rapid direct detection using NA–based methods may be utilized to aid in the diagnosis of infection due to these organisms because they are difficult to culture in the laboratory (Table 18-20).[2,3,141-143] In addition, the diagnosis of infection due to *Bordetella pertussis* (pertussis or whooping cough) can be established utilizing culture (more sensitive if performed within two weeks of the onset of symptoms), serology (not useful in infants and the elderly; utilized late in course of illness), and PCR (useful for diagnosis in patients with symptoms greater than two weeks).[103-105] Also, several rapid direct detection methods using NA–based methods are available for the detection of respiratory viruses and bacteria capable of causing upper and lower respiratory tract infections: Verigene Respiratory Pathogens Flex Test (Luminex, Austin, TX) can detect three *Bordetella* spp. and 13 viral targets including adenovirus, influenza, parainfluenza, rhinovirus, and RSV; Prodesse ProFlu+ Assay (Hologic-Gen-Probe, San Diego, CA) can detect influenza A, influenza B or RSV; xTAG Respiratory Viral Panel (Luminex, Austin TX) can simultaneously detect influenza, RSV, human metapneumovirus, or adenovirus from nasopharyngeal swabs; FilmArray Respiratory Panel (BioFire Diagnostics, Salt Lake City, UT) can simultaneously detect four types of coronavirus, adenovirus, five types of influenza, rhinovirus, parainfluenza, enterovirus, RSV, human metapneumovirus, *B. pertussis, M. pneumoniae*, and *C. pneumoniae* in one hour from a respiratory sample).[141]

In patients with HAP or VAP, semiquantitative analysis of tracheal aspirates or sputum cultures or a quantitative analysis from specimens obtained during BAL may be performed to differentiate between infection and colonization based on the history of prior antibiotic use in the patient and the number of organisms recovered in the sputum specimen.[19] Diagnostic thresholds for pneumonia based on colony counts recovered from a quantitative BAL specimen may differ among institutions. Studies evaluating quantitative BAL or PSB specimens for the diagnosis of HAP or VAP utilize a diagnostic threshold between 10^3–10^5 cfu/mL of an organism for the diagnosis of pneumonia.[2,19,141]

Genitourinary Tract Infections

Urinary Tract Infections

Urinary tract infections (UTIs) are common infections, prompting more than 8 million office visits and over 100,000 hospitalizations per year.[144-147] UTIs are especially common in females due to the close proximity of the urethra (which is shorter than males) to the perirectal and vaginal regions, which are both colonized with bacteria. Because of this anatomic difference, bacteria are able to easily ascend the urethra in females and potentially cause infection in the bladder (cystitis) and upper urinary tract (pyelonephritis). In addition, hospitalized patients (male and female) with indwelling urinary catheters are at increased risk for developing UTIs, with approximately 20% of catheterized patients developing a UTI even with only short-term catheterization.[145,148]

Under normal circumstances, urine within the bladder is sterile because all anatomic sites within the urinary tract above the urethra are not colonized with bacteria. However, the urethra is colonized with bacteria. If noninvasive urine collection methods are utilized for specimen collection, urine will travel through the urethra and may inadvertently collect bacteria as it passes through this nonsterile environment. Therefore, diagnostic criteria have been developed to discriminate between infection, bacterial colonization, or bacterial contamination based on quantitative bacterial colony counts from urine cultures and the presence of inflammatory cells and epithelial cells in the urinalysis. [144-146]

Urine samples for urinalysis and culture can be collected a number of ways. The most common method involves the collection of a clean-catch, midstream urine sample. Before obtaining the sample, the patient should be instructed to clean and rinse the periurethral area with a mild detergent, and then retract the labial folds or penile foreskin when beginning to urinate. The patient should attempt to collect the urine in a sterile cup at the midpoint of the urine stream, collecting the urine sample a few seconds after the start of urination.

Other methods for specimen collection involve invasive procedures such as obtaining urine via bladder catheterization (straight cath) or via suprapubic bladder aspiration. Both of these methods avoid the potential contamination of the urine specimen by the urethra because the urine is collected directly from the bladder. In hospitalized patients with indwelling urinary catheters, urine specimens should be collected directly from the urinary catheter by aspirating the catheter port or tubing (representing freshly voided urine) rather than obtaining the specimen from the collection bag (urine collected over a period of time).[4,144,145,148]

In all cases, urine samples should be immediately transported to the laboratory for processing. Urine samples from patients with acute uncomplicated cystitis may be analyzed only using screening tests such as reagent strip testing (dipsticks), while samples from other patients with UTIs (e.g., recurrent UTIs, pyelonephritis, and UTIs in patients with indwelling catheters) may be analyzed using microscopic examination *and* culture.

Urine samples from women with acute uncomplicated cystitis are usually only evaluated using screening tests because the results are rapidly available and are very useful at excluding the presence of a UTI.[144] The most common rapid screening tests include commercially available reagent test strips, or urine dipsticks, that contain the leukocyte esterase test and the nitrate reductase test, and provide a negative predictive value of 98%.[144,145] The leukocyte esterase test detects the presence of leukocyte esterase, which is an enzyme found in neutrophils. The nitrate reductase test detects the presence of urinary nitrite produced by the reduction of nitrate by nitrate-reducing enzymes of common urinary tract pathogens.[144-146] Positive results from either the leukocyte esterase test or nitrate reductase test warrant treatment for a UTI without the need for urine culture in women with acute uncomplicated cystitis.

The urine from patients with recurrent UTIs, complicated UTIs, or catheter-associated UTIs is typically evaluated using a urinalysis (microscopic examination) and urine culture. The urinalysis is a rapid test that involves the macroscopic and microscopic examination of the urine sample for color, clarity, specific gravity, and the presence of protein, glucose, red blood cells (RBCs), WBCs, bacteria, and epithelial cells. The urinalysis is performed either manually or through the use of automated instruments. Urinalysis findings suggestive of a UTI include specimen cloudiness and the presence of pyuria (>10 WBC/mm^3).[144-146] The detection of pyuria, hematuria, proteinuria, or bacteriuria in the urinalysis may be an indication of infection, but none of these alone is specific for infection. The presence of squamous epithelial cells (>2–5 epithelial cells/mm^3) in a urine sample suggests poor specimen collection and possible contamination.

The urine culture remains the hallmark laboratory test for the diagnosis of UTIs, with quantitative cultures providing the most useful data for determining the clinical significance of isolated bacteria. To establish the diagnosis of a UTI, urine cultures from midstream urine samples should display >10^5 cfu/mL of a single potential uropathogen with concomitant pyuria on urinalysis; however, some women with symptomatic cystitis may have lower colony counts of bacteria (10^3).[145] Colony counts of >10^3 cfu/mL with pyuria are considered clinically relevant in urine specimens from patients with indwelling urethral catheters, intermittent catheterization, men, or children.[144,147] Urine specimens obtained by suprapubic aspiration that display >10^2 cfu/mL with pyuria are indicative of the presence of infection.[144,145,148]

Prostatitis

Bacterial *prostatitis* can present as an acute or chronic infection that typically occurs in males over 30 years of age.[146] The diagnosis of acute bacterial prostatitis is often based on the clinical presentation and the presence of bacteria in a urine specimen. Digital palpation of the prostate and prostatic massage to express purulent secretions are not recommended for the diagnosis of acute bacterial prostatitis because it may induce bacteremia. Conversely, the diagnosis of chronic bacterial prostatitis often cannot be established based on clinical grounds alone because the symptoms are nonspecific and the prostate is often not acutely inflamed. Therefore, chronic prostatitis is classically established through the analysis of sequential urine and prostatic fluid cultures.[146,149] Initially, two samples of urine are obtained for culture—one sample on initiation of urination (VB-1) and one sample obtained at midstream (VB-2). Next, prostate fluid is obtained for culture by massaging the prostate to produce expressed prostatic secretions (EPS). Lastly, a urine sample (VB-3) is obtained after prostatic secretions have been obtained and sent for culture. The diagnosis of chronic bacterial prostatitis is made when the EPS sample contains greater than 10 times the quantity of bacteria cultured from VB-1 or VB-2, or if the VB-3 contains 10 times the quantity of bacteria cultured from VB-1 or VB-2.[146,149] An abbreviated 2-glass specimen method is commonly used in clinical practice and is described in Chapter 23.

Sexually Transmitted Diseases

Gonorrhea

Infection due to *N. gonorrhoeae* is the second most common notifiable sexually transmitted disease (STD) reported in the United States, with most infections involving the mucosa of the cervix, the urethra, the rectum, and the pharynx.[150,151] Infections caused by *N. gonorrhoeae* include localized, uncomplicated, or complicated genital infections (e.g., urethritis, cervicitis, endometritis, pelvic inflammatory disease [PID] in women, and urethritis or epididymitis in men), pharyngitis, anorectal infections, and disseminated infection (e.g., septic arthritis and meningitis) in both men and women.[150,151] Women with genital tract infection and patients with pharyngeal infection are often asymptomatic, while men with urethritis often display symptoms of dysuria and urethral discharge. In addition, patients with *N. gonorrhoeae* are often coinfected with other STDs, such as *Chlamydia trachomatis,* syphilis, or *Trichomonas vaginalis;* therefore, the diagnosis and treatment of all possible STDs in the patient and their sexual partners are important considerations in the control of STDs.[150]

The diagnosis of infection due to *N. gonorrhoeae* can be established using stained clinical smears, culture, or nonculture techniques (only NA amplification tests [NAATs] are currently recommended for routine use; EIA and DNA probe tests are no longer recommended) of urethral, endocervical or urine specimens (NAATs only) that detect cellular components of *N. gonorrhoeae.*[4,150-153]

A presumptive diagnosis of gonorrhea can be made using direct microscopic examination of a clinical specimen using a Gram stain and oxidase test, where gram-negative, oxidase-positive diplococci are demonstrated.[150-153] In addition, the presence of neutrophils on a Gram stain of a urethral specimen is also helpful in establishing the presumptive diagnosis of urethritis.[151] The Gram stain is both sensitive and specific for the presumptive diagnosis of *N. gonorrhoeae* as a point-of-care test for symptomatic men with urethral discharge but is not as useful as a single diagnostic test in asymptomatic men or when evaluating endocervical or pharyngeal specimens.[150,151] Additional tests, such as culture, should be performed to confirm the identification of the organism.

Culture on selective media remains the diagnostic standard for the identification of *N. gonorrhoeae.*[151,153] Culture is recommended for the diagnosis of gonorrhea from urethral, endocervical, vaginal, pharyngeal, or rectal swab (plastic or wire shafts with rayon, Dacron or calcium alginate tips) specimens and should be performed on specimens from all patients (and sexual partners) with suspected gonococcal infections.[151] Culture is also used as a confirmatory test in patients who have suspected gonorrhea based on positive-stained smears or nonculture tests if the specimen has been adequately maintained. However, culture is not optimal in all circumstances due to the tenuous viability of the organism during storage and transport, which prompted the development of nonculture tests for the detection of gonorrhea.[151] Occasionally, susceptibility testing is performed on *N. gonorrhoeae* isolates, especially in patients with suspected or documented treatment failure, to guide the choice of antibiotic therapy, as well as for epidemiologic purposes.[150] In either case, patients are typically given empirical therapy with an antibiotic that demonstrates excellent activity against gonorrhea, keeping in mind that the incidence of β-lactamase-producing, penicillin-resistant gonococci is increasing.

Nonculture tests available for the detection of *N. gonorrhoeae* (even in nonviable organisms) include NAATs, which are able to amplify organism-specific DNA sequences, and the NA hybridization (probe) test, which hybridizes any complementary rRNA that is present in the specimen (cannot differentiate organisms).[150,151] Several NAATs for the detection of *N. gonorrhoeae* are commercially available, and have been designed to detect RNA or DNA sequences using amplification techniques. These tests have been FDA-approved for the detection of *N. gonorrhoeae* in endocervical and vaginal swabs from women, urethral swabs from men, and urine samples from men and women; and because the tests are different, the product information for each individual test should be consulted to dictate the collection methods and clinical specimen type that is suitable for each test.[150,151] These tests are also useful for the detection of *N. gonorrhoeae* from clinical specimens that have not been adequately maintained during transport or collection for culture methods to be utilized.

Chlamydia

Chlamydia trachomatis is the most frequently reported infectious disease in the United States, with >1.3 million cases reported to the CDC in 2010.[150,151] Infection with *C. trachomatis* is now a reportable communicable disease in the United States, with the highest prevalence in persons aged ≤24 years.[150] *C. trachomatis* can cause a number of infections including cervicitis, endometritis, and PID in women; and urethritis, epididymo-orchitis, prostatitis, and proctitis (via receptive anal intercourse) in men.[150] Infection with *C. trachomatis* is also thought to contribute to female infertility and ectopic pregnancies. It is estimated that over $500 million is spent annually on the direct costs associated with the management of *C. trachomatis* infections.[151]

The majority of patients with chlamydial infections are asymptomatic, so that screening is necessary to detect the presence of the organism.[150,151] Because of the asymptomatic nature of chlamydia, it is thought that the current rates of reporting underestimate the true incidence of infection due to this organism. Chlamydia screening is now recommended annually in all sexually active women aged <25 years and other women at increased risk for infection (i.e., new sexual partner, multiple sexual partners, sexual partner with an STD, etc.).[150] In addition, chlamydia screening is also recommended in patients with other STDs because chlamydia often coexists with other STD pathogens.

Culture and nonculture methods are available for the detection of chlamydia. Culture involves the inoculation of the biologic specimen onto a confluent monolayer of cells that support the growth of C. trachomatis, which are then evaluated at 48–72 hours for characteristic intracellular inclusions when infected by *C. trachomatis* that can be detected using a fluorescent monoclonal antibody stain.[151] Cell culture is not routinely utilized by most laboratories due to lack of standardization, technical difficulty, cost, and length of time to yield results (at least 48 hours). Therefore, other nonculture approaches for the laboratory diagnosis of chlamydia have been developed, including the direct fluorescent antibody (DFA) test (not used) and NAATs.

The DFA test involves the staining of a biologic specimen with a fluorescein-labeled monoclonal antibody that binds to *C. trachomatis*-specific antigens (elementary bodies). If the patient is infected with *C. trachomatis*, the antibodies will react with the elementary bodies of the chlamydia in the secretions to produce fluorescence.

The other nonculture test used for the detection of *Chlamydia trachomatis* is the NAAT, which has largely replaced tissue culture and DFA testing because of greater sensitivity and specificity.[150,151,153] There are a number of commercially available NAATs for the detection of *C. trachomatis* that have been designed to detect RNA or DNA sequences using PCR, ligase chain reaction, and various amplification techniques. These tests have been FDA-approved for the detection of *C. trachomatis* in endocervical or vaginal swabs from women, urethral swabs from men, and rectal swab or first catch urine samples from men and women.[150,151]

Syphilis

The spirochete, *T. pallidum,* is the causative pathogen of an STD known as *syphilis*. There are a number of clinical manifestations and stages of syphilis that are based primarily on presenting symptoms and the natural history of the infection[150,153,154]:

1. **Primary syphilis**—characterized by painless ulcers called *chancres* that are typically located at the site of inoculation or initial infection (usually in genital area) and spontaneously resolve over one to eight weeks.
2. **Secondary syphilis**—characterized by systemic symptoms including fever, weight loss, malaise, headache, lymphadenopathy, and a mucocutaneous skin rash (generalized or localized, often involving the palms or the soles of the feet) resulting from hematogenous or lymphatic spread of the organism. If untreated, the manifestations resolve within four to ten weeks.
3. **Latent syphilis**—occurs after secondary syphilis where the organism is still present, but the patient is without symptoms. Latent syphilis acquired within the preceding year is categorized as early latent syphilis, while syphilis acquired greater than one year ago or of unknown duration is categorized as late, latent syphilis. This subclinical infection can be detected only by serologic tests.

4. **Late/tertiary syphilis**—occurs in approximately 35% of untreated patients up to 10–25 years after initial infection; manifested as progressive disease involving the ascending aorta and CNS (neurosyphilis).

T. pallidum cannot be grown in culture; therefore, the diagnosis of syphilis involves the direct detection of the spirochete in biologic specimens by microscopy or the detection of treponemal-specific antibodies using serologic testing.

Direct detection methods can be performed on an appropriate clinical specimen obtained from suspicious genital or skin lesions, including lesion exudate or tissue. The direct detection of *T. pallidum* using dark-field microscopy involves the immediate examination (within 20 minutes of collection) of the biologic specimen under a microscope with a dark-field condenser, looking for the presence of motile spirochetes, where *T. pallidum* can be visualized as 8–10 μm, spiral-shaped organisms.[4,153,154] Another test for the direct detection of *T. pallidum* is the direct fluorescent antibody (DFA-TP) test where the biologic specimen is combined with fluorescein-labeled monoclonal or polyclonal antibodies specific for *T. pallidum* and examined by fluorescence microscopy.[150,154] The interaction between the antibodies and treponemal-specific antigens will produce fluorescence that can be visualized using microscopy.

There are two types of serologic tests that are utilized for the diagnosis of syphilis. The first measures the presence of *nontreponemal* or reaginic antibodies, such as the Venereal Disease Research Laboratory (VDRL) test and the RPR test.[154] The other type of serologic test measures the presence of *treponemal* antibodies, through the use of the fluorescent treponemal antibody absorption (FTA-ABS) test and the microhemagglutination *T. pallidum* (MHA-TP) test.[150,153,154] The use of only one type of serologic test is not sufficient for the diagnosis of syphilis (may result false negative or false positive diagnoses), so persons with a reactive nontreponemal test should undergo treponemal antibody testing to confirm the diagnosis.[150]

The nontreponemal antibody tests (VDRL, RPR) measure the presence of reagin, an antibody-like protein produced in patients with syphilis. However, reagin is also produced in patients with other illnesses including autoimmune diseases, leprosy, TB, malaria, and injection drug use, so false-positive RPR results may occur.[153] Both the RPR and VDRL tests are flocculation tests in which visible clumps are produced in the presence of the reagin antibody (*T. pallidum*) in the submitted specimen. For the VDRL test, the biologic specimen (serum, CSF) is combined with cardiolipin-lecithin coated cholesterol particles on a glass slide and examined microscopically.[154] If the reagin antibody is present in the biologic specimen, visual clumping will occur and be reported as reactive (medium and large clumps). This test can also be quantified by evaluating dilutions of the biologic specimen for reactivity, with the dilution that produces a fully reactive result being reported as the VDRL titer (e.g., 1:8 or 1:32). Therefore, the VDRL titer can be utilized to monitor a patient's response to therapy. The high titers present in untreated disease (e.g., 1:32) traditionally decrease fourfold by 6–12 months and become undetectable in 1–2 years.

The RPR test is a modification of the VDRL test and is commercially available as a reaction card. Sera from the patient is placed on the reaction card and observed for clumping. The RPR result is quantified by evaluating dilutions of the biologic specimen for reactivity, with the highest dilution that produces a fully reactive result being reported as the RPR titer (e.g., 1:8 or 1:32). The RPR titer is also utilized to monitor a patient's response to therapy, where a fourfold decline in titer 6–12 months after therapy would be suggestive of response. The RPR is easier to perform than the VDRL and is used by many laboratories and blood banks for routine syphilis screening. However, the RPR should not be used for the analysis of CSF specimens.

The nontreponemal antibody detection tests are nonspecific when used for screening for the presence of syphilis. Because they are relatively nonspecific, they are most useful for *screening* for the presence of syphilis.[15] False-positive (up to 1–2%) results can occur.[3] In addition, because it takes several weeks for the development of reagin antibodies, false-negative results (up to 25% of patients with primary syphilis) can occur in the early stages of the disease. A positive result from the RPR or VDRL test should be confirmed with the FTA-ABS or the MHA-TP test, which both measure the presence of treponemal-specific antibody.

In the FTA-ABS test, the patient's serum or CSF is initially absorbed with non-*T. pallidum* antigens to reduce cross-reactivity and then applied to a slide on which *T. pallidum* organisms have been fixed followed by addition of a fluorescein-conjugated antihuman antibody for detection of specific antitreponemal antibodies. The amount of fluorescence is subjectively measured by the laboratory technician and reported as reactive, minimally reactive, or nonreactive. Therefore, this test is difficult to standardize among different laboratories. Because this test is also fairly expensive, it is primarily used to verify the results of a positive VDRL or RPR, rather than as a routine screening tool.[154] The FTA-ABS test can detect antibodies earlier in the course of syphilis than nontreponemal tests and, once positive, will remain positive for the life of the patient.

The MHA-TP test is performed using erythrocytes from a turkey, sheep, or other mammal that have been coated with treponemal antigens. These erythrocytes are then mixed with the patient's serum and observed for agglutination, which signifies the presence of antibodies directed against *T. pallidum*. The results are reported as reactive (positive) or nonreactive (negative). Lastly, EIA tests and PCR-based tests for the detection of *T. pallidum* are being evaluated as screening or confirmatory tests for the diagnosis of syphilis, especially for patients where serologic testing is not reliable.[153]

Trichomonas

Infection caused by the protozoan, *T. vaginalis,* is the most common, nonviral STD in the United States, affecting 3.7 million people.[150] *T. vaginalis* is typically diagnosed through detection of actively motile organisms during microscopic examination of wet mount preparations of vaginal secretions, urethral discharge, prostatic fluid, or urine sediment.[114,118,150,153] Because the sensitivity of the wet mount preparation is 50–80%, other diagnostic tests have been developed for the detection of *T. vaginalis* to enhance diagnostic yield, sensitivity, and specificity.[114,118,150,153] Culture using Diamond's medium was considered the diagnostic gold standard and is associated with >80% sensitivity; however, culture methods require proper collection and rapid inoculation for best results, so it is not routinely performed by most laboratories.[118] Several rapid antigen detection methods are commercially available for the diagnosis of infection due to *T. vaginalis* that are easy to perform and employ different assays (IFA and capillary flow ICA).[114,118] Lastly, NA detection methods are highly sensitive and specific tests for the detection of *Trichomonas* and include direct DNA probe (Affirm VPIII, BD, Franklin Lakes, NJ), APTIMA *T. vaginalis* Assay (Hologic-GenProbe, San Diego, CA), BD Probe Tec TV Q^x Amplified DNA Assay and PCR (Amplicor, Roche Diagnostics, Indianapolis, IN) tests.[114,118,150]

Herpes Simplex Virus

Herpes simplex virus (HSV) is the most common cause of genital ulceration in the United States.[153] There are two serotypes of HSV that cause infection: HSV type 1 (HSV-1), most often associated with oropharyngeal infection (cold sores), and HSV type 2 (HSV-2), most commonly associated with genital tract infection. Some patients with genital herpes are asymptomatic, while others experience recurrent vesicular and ulcerative genital lesions, typically at the site of initial infection. In either case, HSV can be transmitted to others by direct contact with virus in secretions from lesions during primary infection or during reactivation of the infection.

In many patients, the diagnosis of HSV is made based on characteristic findings during physical examination, which may include painful vesicles, vesicles that have evolved into pustules, and shallow ulcers with an erythematous base. If lesions compatible with HSV infection are present, the diagnosis can be confirmed by performing a Tzanck smear of a specimen obtained by scraping the base of an active lesion, and visualizing the specimen for characteristic viral intranuclear inclusions using microscopy (cytology and histology).[65,155] Viral culture is the gold standard for the diagnosis of HSV and can also be performed on the specimen from the active lesion; however, it may take up to five to seven days for the virus to grow and be identified, and is associated with low sensitivity, especially in recurrent lesions or as ulcers begin to heal .[150]

Other tests that can be used for the diagnosis of HSV infection include HSV antigen detection (e.g., DFA, indirect fluorescent antibody, immunoperoxidase staining, and EIA); HSV DNA detection by ISH; and HSV DNA-PCR (test of choice for diagnosis of CNS infection and neonatal herpes).[65,150,155] The results from these tests are often available sooner than traditional cell culture and are most useful when rapid detection of HSV is necessary, such as for the diagnosis of encephalitis or before an impending birth.[153] Because viral shedding of HSV is intermittent, negative PCR or culture tests do not exclude the presence of infection, especially in patients without active lesions.[150]

In addition, there are commercially available, FDA-approved serology kits for the diagnosis of HSV-1 and HSV-2 that detect antibody produced against specific HSV antigens. Herpes simplex virus serologic tests are primarily used for the diagnosis of patients with a clinical diagnosis of HSV without laboratory confirmation; for patients with known or suspected exposure to HSV; for patients with recurrent genital symptoms or an atypical presentation with negative HSV PCR or culture, and for determining the serostatus of pregnant women or patients undergoing organ or bone marrow transplantation.[150,156]

Assessing Sterile Body Fluids for the Presence of Infection

Sterile body fluids such as pericardial fluid (pericarditis), pleural fluid (empyema), synovial fluid (septic arthritis), and peritoneal fluid (peritonitis) can be analyzed for the presence of infection. The specimens should be aseptically obtained by needle aspiration, placed in sterile collection tubes, and immediately transported to the laboratory for fluid analysis and culture. Approximately 1–5 mL of fluid should be obtained when analyzing pericardial, pleural, or synovial fluid, while up to 10 mL of peritoneal fluid is required for the diagnosis of peritonitis.[157] All sterile fluids should be processed for cell count (establishing the presence of WBCs with differential), chemistry (protein and glucose), direct microscopic examination including Gram stain (presence of bacteria), and culture. For pleural and synovial fluids, specific criteria are available to aid in the diagnosis of infection (**Table 18-22** and **Table 18-23**).[158-163] Peritoneal fluid characteristics that may be suggestive of peritonitis include a WBC of >250 cells/mm^3, a lactate concentration >25 mg/dL, a pH <7.35, a fluid:blood glucose ratio of <0.7 (in TB peritonitis), and an elevated protein concentration (except in cirrhotic patients).[164] The diagnosis of infection in each of these sites should be established based on the presence of WBCs and other characteristic chemistry abnormalities in the sterile fluid specimen, the growth of a pathogenic organism from the cultured material, and the characteristic signs and symptoms of infection at the infection site.

ACUTE PHASE REACTANTS AND INFECTION

Chapter 19 provides information on the background, normal range, and clinical utility of acute phase reactants such as the ESR and the CRP as they relate to the diagnosis of inflammatory diseases. The ESR and CRP may also be elevated in the presence of infection.[165-171] Elevations in the ESR and CRP do not differentiate between inflammatory or infectious processes because they increase in response to tissue injury of any cause. However, the ESR and CRP are often elevated in the presence of infection—with increased levels reported in

TABLE 18-22. Pleural Fluid Findings and Interpretation[158–160]

	TRANSUDATIVE (SUGGESTIVE OF CHF, CIRRHOSIS)	EXUDATIVE (SUGGESTIVE OF INFECTION SUCH AS EMPYEMA, MALIGNANCY, PANCREATITIS WITH ESOPHAGEAL PERFORATION, SLE)
Appearance	Clear, serous	Cloudy
pH	>7.2	<7.2
LDH (IU/L)	<200	≥200
Pleural fluid LDH to serum LDH ratio	<0.6	>0.6
Protein (g/dL)	<3	>3
Pleural fluid to serum protein ratio	<0.5	>0.5
Glucose (mg/dL)	>60 (same as serum)	<40–60
WBCs (count/mm^3)	<10,000	>10,000
WBC differential	<50% PMNs	If infectious, depends on pathogen

CHF = congestive heart failure; LDH = lactate dehydrogenase; PMNs = polymorphonuclear leukocytes; SLE = systemic lupus erythematosus; WBC = white blood cell.

TABLE 18-23. Synovial Fluid Findings and Interpretation[161-163]

	NORMAL	NONINFLAMMATORY (OSTEOARTHRITIS, TRAUMA, AVASCULAR NECROSIS, SLE, EARLY RHEUMATOID ARTHRITIS)	INFLAMMATORY (RHEUMATOID ARTHRITIS, SPONDYLOARTHROPATHIES, VIRAL ARTHRITIS, CRYSTAL-INDUCED ARTHRITIS)	PURULENT (BACTERIAL INFECTION, TUBERCULOUS INFECTION, FUNGAL INFECTION)
WBCs (count/mm^3)	<150	<3000	3000–50,000	>50,000
WBC differential	No predominance	<25% PMNs	>70% PMNs, variable	>75–90% PMNs
Protein (g/dL)	1.3–1.8	3–3.5	>3.5	>3.5
Glucose (mg/dL)	Normal	Normal	70–90	<40–50

PMNs = polymorphonuclear leukocytes; SLE = systemic lupus erythematosus; WBC = white blood cell.

bacterial otitis media, osteomyelitis, endocarditis, PID, and infections in transplant patients—and may serve as an adjunctive modality to aid in the diagnosis of these infections.[165-171] Serial measurement of the ESR, and especially the CRP, may also be useful in assessing the response to antibiotic therapy in the treatment of deep-seated infections such as endocarditis or osteomyelitis.[165-171]

Procalcitonin is the precursor of calcitonin, a calcium regulatory hormone, which is also an acute phase reactant that is produced in response to systemic inflammation because it is thought to represent the activation of innate immunity in response to invasion by bacteria, malaria, and some fungi (not viruses).[165,172-174] Procalcitonin levels become detectable within 2–4 hours of infection and peak at 6–24 hours, with the extent of production correlating with bacterial load and severity of infection.[172,173] It was originally believed that procalcitonin levels increased in response to tissue injury or sepsis induced only by infection; however, levels of procalcitonin may be elevated in other inflammatory diseases or situations such as autoimmune diseases, severe trauma, cirrhosis, pancreatitis, burns, and hypotension during surgery.[165,172-174] The use of procalcitonin in the diagnosis of infection has been evaluated in numerous studies involving different patient types, infections and clinical settings , with several procalcitonin-based algorithms being developed to (1) determine the presence of infection or guide initiation of antibiotic therapy, (2) evaluate the efficacy of empiric antibiotic therapy, and (3) determine when antibiotic therapy can be deescalated or discontinued during the treatment of an infection.[172,173] Procalcitonin levels that correlate with the presence of infection have not been clearly defined for all infection types and clinical settings, but it does appear as if procalcitonin levels <0.1 mcg/L exclude the presence of infection.[173] Additional research is needed to further define the role of procalcitonin in the diagnosis and management of different patient types, infections, and clinical settings.[172-174]

SUMMARY

Although infectious disease is a rapidly changing field because of new challenges and technological advances, the diagnosis of many infectious illnesses depends on proper performance and interpretation of numerous basic laboratory tests. For example, the Gram stain is a readily available, invaluable tool for examining clinical specimens for the presence of bacteria. Culture of clinical specimens using appropriate growth media allows for the cultivation and identification of many infecting bacteria, which often takes 24–48 hours. However, numerous rapid diagnostic tests are now available for the identification of bacteria directly from clinical specimens such as blood, stool, respiratory secretions, or body fluids, which substantially decrease the time to organism identification when compared to traditional bacterial culture and identification methods.[3] Susceptibility tests for rapidly growing aerobic bacteria are commonly performed using an automated microdilution or a manual disk diffusion method. Bacterial susceptibilities to various antimicrobial agents are reported as S, I, and R. National standards for susceptibility testing are available and help guide the performance of the tests, the choice of antimicrobial agents to evaluate for susceptibility, and the reporting procedures of susceptibility tests by the clinical microbiology laboratory. Empiric antimicrobial therapy is typically chosen based on the suspected site and subsequent potential causative organisms of infection (using local or regional susceptibility information). Once the results of bacterial culture and susceptibility testing are available, antimicrobial therapy is deescalated, if possible, to a more targeted (directed) regimen based on the susceptibility profile of the infecting organism in conjunction with patient-specific (e.g., clinical condition, site of infection, drug allergies, and renal function) and infection-specific information.

New testing methods and guidelines have recently become available for the recovery, identification, and susceptibility testing of fungi, mycobacteria, and viruses. These processes may challenge the clinical microbiology laboratory due to their requirements for specialized staining, culturing, and susceptibility testing procedures.

Lastly, several infection types (e.g., meningitis, UTIs) and certain pathogens (e.g., *B. burgdorferi* and *L. pneumophila*) often require specialized laboratory testing to aid in the identification of the infecting organism. The clinician should be aware of the diagnostic tests currently available for these infections.

LEARNING POINTS

1. ***What methods do most microbiology laboratories in the United States use to perform antimicrobial susceptibility testing, and how is this information conveyed to the clinician?***

   ANSWER: Microbiology laboratories often utilize several methods for antimicrobial susceptibility testing to accurately determine the activity of antibiotics against many different types of bacteria (e.g., aerobic, anaerobic, and fastidious). However, most laboratories predominantly use automated broth microdilution methods (Vitek 2, MicroScan) that utilize commercially prepared, disposable microtiter trays/cassettes for antimicrobial susceptibility testing that can test the susceptibility of multiple antibiotics simultaneously, while decreasing cost and labor. Although many microbiology laboratories are performing rapid diagnostic tests for the identification of bacteria and the detection of pertinent resistant gene markers, antimicrobial susceptibility testing (using automated microdilution methods) is still being performed to determine the exact susceptibility/resistance profile of the infecting bacteria. The antimicrobial susceptibility results for each bacteria are compiled in a report that contains the following information: antibiotic tested, MIC or MIC range (especially with automated broth microdilution methods), and CLSI interpretive criteria (S, I, and R). These reports are usually located in the patient's medical chart (electronic or paper) and in the hospital/laboratory information system. Refer to Minicase 2 for a specific example of an antimicrobial susceptibility report.

2. ***What laboratory tests are utilized in the diagnosis of HIV infection? What surrogate laboratory markers are used to assess the immunocompetence of patients infected with HIV? What laboratories are available to detect HIV resistance to available antiretrovirals?***

   ANSWER: Current recommendations for the diagnosis of HIV infection include the initial use of a fourth-generation antibody/antigen combination ELISA (or EIA) followed by a supplemental HIV-1/HIV-2 antibody differentiation immunoassay when the initial test is reactive (positive), and the use of NA tests when the antibody differentiation immunoassay is nonreactive or indeterminate. The CD4 cell count and the plasma HIV viral load are the two surrogate laboratory markers that are routinely used throughout the course of HIV infection to assess the immunocompetence of patients infected with HIV and are often used to determine the indications for antiretroviral treatment as well as to monitor the effectiveness of antiretroviral therapy. HIV genotype assays sequence or probe genes in specific areas of the virus where different classes of antiretrovirals exert their action. This type of assay is recommended after HIV diagnosis, before starting antiretroviral therapy, and when HIV resistance is suspected based on unresponsiveness of the viral load despite good adherence to therapy.

3. ***What are the major laboratory tests that are used in the diagnosis of the UTIs, meningitis, pneumonia, and septic arthritis?***

   ANSWER: In patients with signs and symptoms suggestive of a UTI, a urine sample (clean-catch midstream, catheterized specimen, suprapubic aspiration) is usually sent to the laboratory for microscopic analysis (urinalysis) and culture. In patients with signs and symptoms suggestive of meningitis, a lumbar puncture is performed to obtain CSF that is evaluated for general appearance, glucose concentration, protein concentration, WBC count, WBC differential, Gram stain, and culture. In addition, depending on the medical history of the patient, specialized tests may also be performed on the CSF. In patients with signs and symptoms suggestive of pneumonia, a sputum sample (expectorated, BAL, PSB) is submitted to the laboratory for adequacy evaluation, Gram stain and culture, and, occasionally, *S. pneumoniae* urinary antigen. In patients with suspected septic arthritis, a synovial fluid aspirate is analyzed for cell count (presence of WBCs with differential), chemistry (protein and glucose), direct microscopic examination including Gram stain (presence of bacteria), and culture. Patients with septic arthritis may also have an elevated ESR or CRP. Also, in all of the above infections, patients may also exhibit leukocytosis and a left shift, which would be demonstrated on a CBC.

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19

RHEUMATOLOGIC DISEASES

Susan P. Bruce and Terry L. Schwinghammer

OBJECTIVES

After completing this chapter, the reader should be able to

- Describe the physiologic basis for rheumatologic laboratory tests and the pathophysiologic processes that result in abnormal test results
- Understand the appropriate clinical applications for laboratory tests used to diagnose or assess the activity of select rheumatologic diseases
- Interpret the results of laboratory tests used to diagnose or manage common rheumatologic diseases
- Use the results of rheumatologic laboratory tests to make decisions about the effectiveness of pharmacotherapy
- Employ laboratory tests to identify and prevent adverse reactions to drugs used to treat rheumatologic diseases

The diagnosis and management of most rheumatologic diseases depend primarily on patient medical history, symptoms, and physical examination findings. A variety of laboratory tests are used to assist in the diagnosis of rheumatologic disorders, but many are nonspecific tests that are not pathognomonic for any single disease. However, the results of some specific laboratory tests may be essential for confirming the diagnosis of some diseases. Consequently, laboratory tests are important diagnostic tools when used in concert with the medical history and other subjective and objective findings. Some laboratory test results are also used to assess disease severity and to monitor the beneficial and adverse effects of pharmacotherapy.

The diagnostic utility of a laboratory test depends on its sensitivity, specificity, and predictive value (Chapter 1). Tests that are highly sensitive and specific for certain rheumatologic diseases often have low predictive values because the prevalence of the suspected rheumatologic disease is low. The most important determinant of a laboratory test's diagnostic usefulness is the pretest probability of disease, or a clinician's estimated likelihood that a certain disease is present based on history and clinical findings. As the number of disease-specific signs and symptoms increases and approaches diagnostic confirmation, the pretest probability also increases.

After briefly reviewing pertinent physiology of immunoglobulins, this chapter discusses various tests used to diagnose and assess rheumatologic diseases, followed by interpretation of these test results in common rheumatologic disorders. Tests used to monitor antirheumatologic pharmacotherapy are also described.

STRUCTURE AND PHYSIOLOGY OF IMMUNOGLOBULINS

Many rheumatologic laboratory tests involve detection of immunoglobulins (antibodies) that are directed against normal cellular components. The structure and functions of immunoglobulins are reviewed briefly here to facilitate understanding of these tests.

When the immune system is challenged by a foreign substance (antigen), activated B lymphocytes differentiate into immunoglobulin-producing plasma cells. Immunoglobulins are Y-shaped proteins with an identical antigen-binding site (called *Fab* or *fraction antigen-binding*) on each arm of the Y (**Figure 19-1**). Each arm is composed of a light (L) amino acid chain covalently linked to a heavy (H) amino acid chain. The terms *light* and *heavy* refer to the number of amino acids in each chain. Because the heavy chain has more amino acids than the light chain, it is longer and has a higher molecular weight.

Both types of chains have a variable region (V_L and V_H) and a constant region (C_L and C_H). The variable regions contain the antigen-binding sites and vary in amino acid sequence. The sequences differ to allow immunoglobulins to recognize and bind specifically to thousands of different antigens. Within the variable regions, there are four framework regions and three complementarity-determining regions; together these make up the antigen-binding pocket. The constant region of the light chain (C_L) is a single section. Immunoglobulins that have identical constant regions in their heavy chains (e.g., C_H1, C_H2, and C_H3) are of the same class.

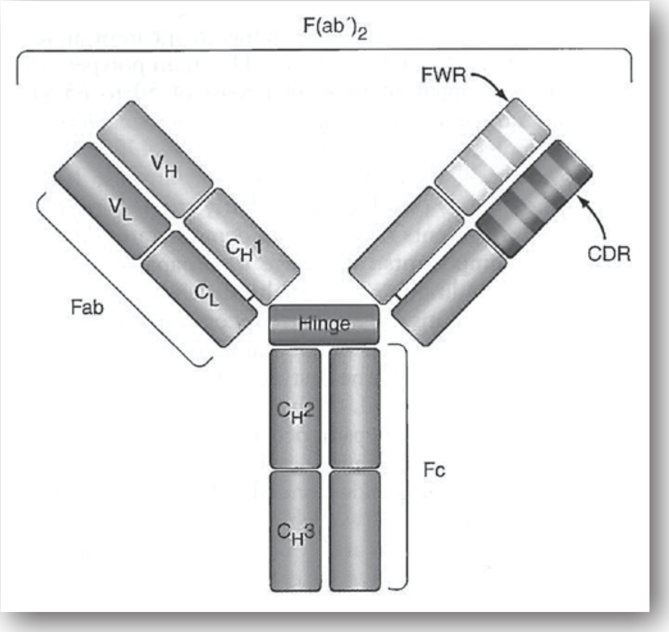

FIGURE 19-1. Schematic of the antibody molecule. (Reproduced, with permission, from Orduno NM, Grimaldi C, Diamond B. B cells. In: Firestein GS, Budd RC, Gabriel S et al., eds. Kelley's textbook of rheumatology. 9th ed. Philadelphia: Saunders Elsevier; 2013;192.)

The five classes of immunoglobulins are IgA, IgD, IgE, IgG, and IgM. Depending on the immunoglobulin, the constant region of the heavy chain has either three domains and a hinge region (IgA, IgD, and IgG) that promotes flexibility, or four domains without a hinge region (IgE and IgM). Thus, the immunoglobulin's heavy chain determines its class (alpha heavy chains, IgA; delta heavy chains, IgD; epsilon heavy chains, IgE; gamma heavy chains, IgG; and mu heavy chains, IgM). Tests are available to measure the serum concentrations of the general types of immunoglobulins as well as immunoglobulins directed against specific antigens (viruses, other infectious agents, other allergens).

In Figure 19-1, the second and third domains (C_H2 and C_H3) of the heavy chain are part of the Fc (fraction crystallizable) portion of the immunoglobulin. This portion has two important functions: (1) activation of the complement cascade (discussed later); and (2) binding of immunoglobulins (which react with and bind antigen) to cell surface receptors of effector cells such as monocytes, macrophages, neutrophils, and natural killer cells.[1]

TESTS TO DIAGNOSE AND ASSESS RHEUMATOLOGIC DISEASES

Blood tests that are relatively specific for certain rheumatologic diseases include rheumatoid factors (RFs), anticitrullinated protein antibodies (ACPAs), antinuclear antibodies (ANAs), antineutrophil cytoplasmic antibodies (ANCAs), and complement. Nonspecific blood and other types of tests include erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), analysis of synovial fluid, and others. Where applicable, the sections that follow discuss quantitative assay results (where normal values are reported as a range of concentrations), qualitative assay results (where assay results are reported as only positive or negative), and their use in common rheumatologic and nonrheumatologic diseases.

Anticitrullinated Protein Antibodies

Citrullination is the process by which susceptible genes exposed to environmental factors undergo an abnormal change. As a result, citrullinated proteins become antigens and lead to the formation of autoantibodies. ACPAs bind to the nonstandard amino acid citrulline that is formed from removal of amino groups from arginine. Nonstandard amino acids are generally not found in proteins and often occur as intermediates in the metabolic pathways of standard amino acids. In the joints of rheumatoid arthritis (RA) patients, proteins may be transformed to citrulline during the process that leads to joint inflammation. The creation of citrullinated proteins is not specific to RA. However, the resulting formation of ACPAs is unique to RA. When these antibodies are present, there is a 90–95% likelihood that the patient has RA. The combination of both positive RF and positive ACPA has 99.5% specificity for RA.

The ACPA test is most useful in helping to identify the etiology of inflammatory arthritis in patients with negative rheumatoid factor (RF) titers. ACPAs are detected in about 50–60% of patients with early RA, usually after three to six months of symptoms.[3] It has been theorized that citrulline antibodies represent the earlier stages of RA in this situation. The presence of ACPAs have also been associated with more erosive forms of RA. The presence of ACPAs is considered one of the clinical features of RA that are associated with a worse long-term prognosis. Therefore, ACPA-positive patients with moderate or high disease activity should receive aggressive treatment early in the course of RA.

Quantitative and Qualitative Assay Results

Normal values: <20 EU/mL (assay dependent)

Quantitative ACPAs are tested by enzyme-linked immunosorbent assay (ELISA) and are reported in ELISA units (EU). The relationship between these values and qualitative results are generally reported as the following:

- <20 EU: negative
- 20–39 EU: weakly positive
- 40–59 EU: moderately positive
- >60 EU: strongly positive

Rheumatoid Factor

Rheumatoid factors (RFs) are immunoglobulins (predominantly IgM but may also be IgG or IgA) that are abnormally directed against the Fc portion of IgG. These immunoglobulins do not recognize the IgG as being "self." Therefore, the presence of RFs in the blood indicates an autoimmune process. The RF measured in most laboratories is IgM-anti-IgG (an IgM antibody that specifically binds IgG). Like all IgM antibodies, IgM RF is composed of five subunits whose Fc portions are attached to the same base. The variable regions of each IgM antibody can bind up to five IgG molecules at its multiple binding sites, making IgM RF the most stable and easiest to quantify.

RFs are most commonly associated with RA but are not specific for that disease. Other rheumatologic diseases in which circulating RFs have been identified include systemic lupus erythematosus (SLE), systemic sclerosis (scleroderma), mixed connective tissue disease (MCTD), and Sjögren syndrome.[2] The significance of RFs in these diseases is unknown.

The presence of RF is not conclusive evidence that a rheumatologic disease exists. Patients with various acute and chronic inflammatory diseases as well as healthy individuals may be RF positive. Nonrheumatologic diseases associated with RFs include mononucleosis, hepatitis, malaria, tuberculosis, syphilis, subacute bacterial endocarditis, cancers after chemotherapy or irradiation, chronic liver disease, hyperglobulinemia, and cryoglobulinemia.

The percentage of individuals with positive RF concentrations and the mean RF concentration of the population increase with advancing age, but is 1–2% of the heathy population on average. Although RFs are associated with several rheumatologic and many nonrheumatologic diseases, the concentrations of RFs in these diseases are lower than those observed in patients with RA.

Quantitative Assay Results

Normal values: <1:16 or <15 IU/mL

When a quantitative RF test is performed, results are reported as either a dilutional titer or a concentration in international units per milliliter (IU/mL). RF titers are reported positive as a specific serum dilution; the ability to detect RF is tested at each dilution. The greatest dilution that results in a positive test is reported as the endpoint. A titer of >1:16 or a concentration >15 IU/mL is generally considered to be positive. However, reference ranges vary depending on the method used, so it is important to use the limits provided by the individual laboratory.

Qualitative Assay Results

The dilutional titer chosen to indicate a positive RF excludes 95% of the normal population. Stated another way, at a serum dilution at which 95% of the normal population is RF negative, 70% to 90% of RA patients will have a positive RF test. The remaining RA patients who have RF titers within the normal range may be described as seronegative.

Antinuclear Antibodies

Antinuclear antibodies (ANAs) are a heterogeneous group of autoantibodies directed against nucleic acids and nucleoproteins within the nucleus and cytoplasm. Intracellular targets of these autoantibodies include deoxyribonucleic acid (DNA), ribonucleic acid (RNA), individual nuclear histones, acidic nuclear proteins, and complexes of these molecular elements (**Table 19-1**).[4-7]

The ANA test is included in the diagnostic criteria for idiopathic SLE, drug-induced lupus, and MCTD because of its high rate of positivity in these disorders. However, its low specificity makes it unsuitable for use as a screening test for rheumatologic or nonrheumatologic diseases in asymptomatic individuals. A positive ANA can also be found in otherwise healthy individuals. ANAs are also associated with various genetic and environmental factors (e.g., intravenous drug abuse), hormonal factors, and increased age. They also are associated with nonrheumatologic diseases, both immunologically mediated (e.g., Hashimoto thyroiditis, idiopathic pulmonary fibrosis, primary pulmonary hypertension, idiopathic thrombocytopenic purpura, and hemolytic anemia) and nonimmunologically mediated (e.g., acute or chronic bacterial, viral, or parasitic infections; and neoplasm).

Antibody tests that have clinical utility for diagnosis of SLE, drug-induced lupus, and other diseases include the following:

- **Double-stranded DNA (dsDNA) antibodies**—These antibodies are relatively specific for SLE, which makes them useful for diagnosis of the disorder. In some patients with SLE, the titers tend to rise with a disease flare and fall (usually into the normal range) when the flare subsides. Thus, dsDNA titers may be helpful in managing disease activity in some SLE patients. The dsDNA antibodies have been found in low titers in many other autoimmune diseases (e.g., RA, Sjögren syndrome, systemic sclerosis, Raynaud phenomenon, MCTD, discoid lupus, juvenile idiopathic arthritis [JIA], and autoimmune hepatitis).[6] The presence of dsDNA antibodies has also been reported in patients receiving some drugs used to treat rheumatologic diseases (e.g., minocycline, etanercept, infliximab, and penicillamine).
- **Single-stranded DNA (ssDNA) antibodies**—These antibodies identify and react primarily with purine and pyrimidine bases within the β-helix of dsDNA. They may also bind with nucleosides and nucleotides. They are much less specific for SLE than dsDNA antibodies. Therefore, ssDNA antibodies are of limited usefulness for diagnosing SLE.[6] They also do not correlate well with disease activity and are not helpful for managing ongoing disease.
- **Smith (Sm) antibodies**—These antibodies bind to a series of nuclear proteins complexed with small nuclear RNAs. These complexes are known as *small nuclear ribonucleoprotein particles* and are important in the processing of RNA transcribed from DNA.[6] The Sm antibody test has low sensitivity (10–50% depending on assay methodology) but high specificity (55–100%) for SLE. Titers usually remain positive after disease activity has subsided and titers of anti-DNA antibodies have declined to the normal range. Thus, the Sm antibody titer may be a useful diagnostic tool, especially when anti-DNA antibodies are undetectable. There is currently no evidence that monitoring Sm antibodies is useful for following the disease course or predicting disease activity.[6]
- **Ribonucleoprotein (RNP) or uridine-rich ribonuclear protein (U_1RNP) antibodies**—This antibody

TABLE 19-1. Laboratory and Clinical Characteristics of Antibodies to Nuclear/Cytoplasmic Antigens

ANA	TARGETED CELLULAR MATERIAL	SENSITIVITY	SPECIFICITY
dsDNA	dsDNA	SLE: 70% RA: 1% Systemic sclerosis: <1%	SLE: high (>95%) Drug-induced lupus: low (1–5%) RA: low (1%) Systemic sclerosis: low (<1%) Sjögren syndrome: low (1–5%)
ssDNA	ssDNA	SLE: 80% Drug-induced lupus: 80% RA: 60%	SLE: low Drug-induced lupus: low RA: moderate
Sm[a]	Nuclear ribonucleoproteins	SLE: 10–50% Drug-induced lupus: 1% RA: 1% Systemic sclerosis: <1% Sjögren syndrome: 1–5%	SLE: high (55–100%)
RNP (or U_1RNP)	Nuclear ribonucleoproteins	MCTD: 100% SLE: 3–69% Systemic sclerosis: <1% RA: 25% Polymyositis: <1%	MCTD: low SLE: low Systemic sclerosis: low RA: low Polymyositis: low
Histone	Chromatin and DNA-packing protein	SLE: 70% Drug-induced lupus: 95% RA: 15–20% Systemic sclerosis: <1%	SLE: moderate Drug-induced lupus: high RA: low Systemic sclerosis: low
Ro[a]/SSA[b]	Nuclear ribonucleoproteins	Sjögren syndrome: 10–60% SLE: 40% Polymyositis: 18% RA: 5% Systemic sclerosis: 5%	Sjögren syndrome: moderate SLE: low Polymyositis: low RA: low Systemic sclerosis: low
La[a]/SSB[b]	Nuclear ribonucleoproteins	Sjögren syndrome: 70–95% SLE: 10–35%	Sjögren syndrome: high (94%) SLE: low
Centromere (ACA)	Chromatin and centromere	Systemic sclerosis: 25–30% CREST: 50–90% Raynaud phenomenon: 15–30%	Systemic sclerosis: high CREST, Raynaud phenomenon: high (>95%)
DNA topoisomerase I (Scl_{70})	Chromatin and DNA-catalyzing protein	Systemic sclerosis: 15–20%	Systemic sclerosis: high (>95%)
Jo-1	Cytoplasm and histidyl tRNA synthetase	SLE: low Drug-induced lupus: low RA: low Systemic sclerosis: low Sjögren syndrome: low Polymyositis: 30% Jo-1 syndrome: 50%	SLE: low Drug-induced lupus: low RA: low Systemic sclerosis: low Sjögren syndrome: low Polymyositis with interstitial lung disease: high Jo-1 syndrome: high

ACA = anticentromere antibody; ANA = antinuclear antibody; CREST = syndrome characterized by calcinosis, Raynaud phenomenon, esophageal motility disorder, sclerodactyly, and telangiectasias; DNA = deoxyribonucleic acid; dsDNA = double-stranded DNA; La/SSB = La/Sjögren syndrome B antibody; MCTD = mixed connective tissue disease; RA = rheumatoid arthritis; RNP = ribonucleoprotein; Ro/SSA = Ro/Sjögren syndrome A antibody; SLE = systemic lupus erythematosus; Scl_{70} = scleroderma-70 or DNA topoisomerase I antibody; Sm = Smith antibody; ssDNA = single-stranded DNA; U_1RNP = uridine-rich ribonuclear protein.

[a] Represents the first two letters of the surname of the patient whose serum was used to identify the reaction in agar diffusion.

[b] Sjögren syndrome A and B.

Source: From references 4–7.

system reacts to antigens that are related to Sm antigens. However, these antibodies bind only to the U_1 particle, which is involved in splicing nuclear RNA into messenger RNA. Ribonucleoprotein antibodies are found in many patients with SLE (3–69%), and low titers may be detected in other rheumatologic diseases (e.g., Raynaud phenomenon, RA, systemic sclerosis).[6] Importantly, RNP antibodies are a hallmark feature of MCTD. A positive test in a patient with suspected MCTD increases the probability that this diagnosis is correct, even though the test is nonspecific. On the other hand, a negative anti-RNP in a patient with possible MCTD virtually excludes this diagnosis.

- **Histone (nucleosome) antibodies**—These antibodies target the protein portions of nucleosomes, which are DNA-protein complexes comprising part of chromatin. These antibodies are present in virtually all cases of drug-induced lupus. In fact, the diagnosis of drug-induced lupus should be questioned in their absence. Most cases of drug-induced lupus are readily diagnosed because a commonly implicated drug (e.g., hydralazine, isoniazid, procainamide) is being taken or a strong temporal relationship exists between drug initiation and the onset of SLE signs and symptoms. However, in some cases of potential drug-induced lupus, histone autoantibody testing can be helpful. Histone antibodies appear less commonly in other diseases, including RA, JIA, autoimmune hepatitis, scleroderma, and others. There is some evidence that histone antibodies correlate with disease activity in SLE.

Two closely related ANA tests are detected frequently in patients with Sjögren syndrome, but they are nonspecific; they may also be helpful for diagnosis of SLE.

1. Ro/Sjögren syndrome A (Ro/SSA) antibody
2. La/Sjögren syndrome B (La/SSB) antibody

The presence of either antibody in patients with suspected Sjögren syndrome strongly supports the diagnosis. It is unusual to detect the La/SSB antibody in patients with SLE or Sjögren syndrome in the absence of the Ro/SSA antibody. In women of childbearing age who have a known connective-tissue disease (e.g., SLE, MCTD), a positive Ro/SSA antibody is associated with an infrequent but definite risk of bearing a child with neonatal SLE and congenital heart block. Presence of the Ro/SSA antibody also correlates with late-onset SLE and secondary Sjögren syndrome. In patients who are ANA negative but have clinical signs of SLE, a positive Ro/SSA antibody may be useful in establishing a diagnosis of SLE.

It has been recommended that the Ro/SSA antibody test be ordered in the following situations[7]: (1) women with SLE or Sjögren syndrome who are planning to become pregnant; (2) women with a history of giving birth to children with heart block or myocarditis; (3) women known to be ANA positive who wish to become pregnant; (4) patients suspected of having a systemic connective tissue disease with a negative ANA screening test; and (5) patients with xerostomia, keratoconjunctivitis sicca, and salivary and lacrimal gland enlargement.

Two ANAs are highly specific for systemic sclerosis (scleroderma), but the tests have low sensitivity:

1. Anticentromere antibody (ACA)
2. DNA topoisomerase I (ScI_{70}) antibody

In addition, these two antibodies are highly specific for CREST syndrome (associated with **c**alcinosis, **R**aynaud phenomenon, **e**sophageal dysmotility, **s**clerodactyly, and **t**elangiectasias), Raynaud phenomenon, and occasionally SLE. When systemic sclerosis is suspected on clinical grounds, antibody testing for ACA and ScI_{70} can be useful in making the diagnosis. However, negative results do not exclude the disease because of low test sensitivity.

The Jo-1 antibody (anti-Jo) is highly specific for idiopathic inflammatory myopathy including polymyositis and dermatomyositis, or myositis associated with another rheumatologic disease or interstitial lung disease. **Figure 19-2** provides guidelines for the use of the ANA test in diagnosing rheumatologic disorders. The titer or quantitative value should be considered when evaluating the clinical significance of ANA test results.

Quantitative Antinuclear Antibody Assay Results

Normal: Negative at 1:40 dilution (varies among laboratories)
The indirect immunofluorescence antinuclear antibody test (FANA) is a rapid and highly sensitive method for detecting the presence of ANAs.[4] It is considered the gold standard for ANA testing. Although the FANA is positive in >95% of patients with SLE, it is also positive in some normal individuals and patients with drug-induced lupus and other autoimmune diseases. An

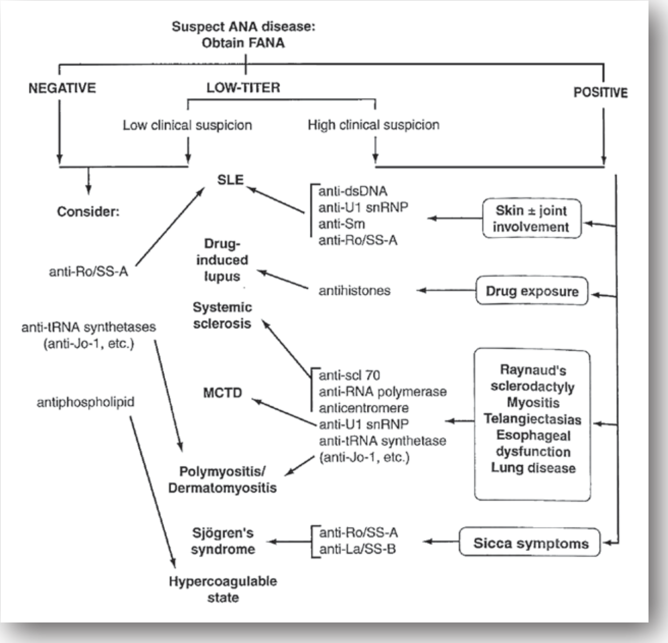
Suspect ANA disease:
Obtain FANA
NEGATIVE
LOW-TITER
POSITIVE
Low clinical suspicion
High clinical suspicion
Consider:
anti-Ro/SS-A
anti-tRNA synthetases (anti-Jo-1, etc.)
antiphospholipid
SLE
Drug-induced lupus
Systemic sclerosis
MCTD
Polymyositis/ Dermatomyositis
Sjögren's syndrome
Hypercoagulable state
anti-dsDNA
anti-U1 snRNP
anti-Sm
anti-Ro/SS-A
antihistones
anti-scl 70
anti-RNA polymerase
anticentromere
anti-U1 snRNP
anti-tRNA synthetase (anti-Jo-1, etc.)
anti-Ro/SS-A
anti-La/SS-B
Skin ± joint involvement
Drug exposure
Raynaud's
sclerodactyly
Myositis
Telangiectasias
Esophageal dysfunction
Lung disease
Sicca symptoms

FIGURE 19-2. Algorithm for the use of ANAs in the diagnosis of connective tissue disorders. (Reproduced with permission from Peng ST, Craft JE. Antinuclear antibodies. In: Firestein GS, Budd RC, Gabriel SE et al., eds. Kelley's textbook of rheumatology. 9th ed. Philadelphia: Saunders Elsevier; 2013;794.)

ELISA also provides a rapid and highly sensitive method for detecting the presence of ANA, however the sensitivity is lower than FANA. Many laboratories perform screening ANA tests by the ELISA technique because it can be automated and is less labor intensive; FANA testing is performed only in specimens testing positive by ELISA.[5] It is important for the clinician to know the technique utilized at a specific laboratory to interpret the results correctly.

Laboratories usually report the ANA titer, which is the highest serum dilution that remains positive for ANAs. A very high concentration (titer >1:640) should raise suspicion for an autoimmune disorder but is not in itself diagnostic of any disease. In the absence of clinical findings, these individuals should be monitored closely for the overt development of an autoimmune disorder. On the other hand, a high ANA titer is less useful in a patient who already has definite clinical evidence of a systemic autoimmune disease. The finding of a low antibody titer (<1:80) in the absence of signs or symptoms of disease is not of great concern, and such patients require less frequent followup than those with very high titers. False-positive ANAs are common in the normal population and tend to be associated with low titers (<1:40). The positive antibody titers in healthy persons tend to remain fairly constant over time; this finding can also be seen in patients with known disease.

Qualitative Antinuclear Antibody Assay Results

The pattern of nuclear fluorescence after staining may reflect the presence of antibodies to one or more nuclear antigens. The nuclear staining pattern was used commonly in the past, but the pattern type is now recognized to have relatively low sensitivity and specificity for individual autoimmune diseases. For this reason, specific antibody tests have largely replaced use of patterns.[5] The common immunofluorescent patterns are as follows:

- **Homogeneous**—This pattern is seen most frequently in patients with SLE but can also be observed in patients with drug-induced lupus, RA, vasculitis, and polymyositis. This pattern reflects antibodies to the DNA-histone complex.
- **Speckled**—This pattern is also seen most frequently in SLE but can appear in patients with MCTD, Sjögren syndrome, progressive systemic sclerosis, polymyositis, and RA. This pattern is produced by antibodies to Sm, Ro/SSA, La/SSB, DNA topoisomerase I (ScI_{70}), and other antigens.
- **Nucleolar**—This pattern is infrequently observed in patients with SLE but is more frequently seen in patients with polymyositis, progressive systemic sclerosis, and vasculitis. It is produced by antibodies to RNA polymerase I and a number of other antigens.
- **Peripheral or nuclear rim**—This is the only pattern that is highly specific for any rheumatologic disease and is observed predominantly (98%) in SLE patients. It is produced by antibodies to DNA (dsDNA, ssDNA) and nuclear envelope antigens (antibodies to components of the nuclear envelope, such as certain glycoproteins).

Table 19-1 summarizes the most frequently identified ANAs, their corresponding targeted cellular material, and disease sensitivities and specificities.[4-7]

Antineutrophil Cytoplasmic Antibodies

As the name implies, *antineutrophil cytoplasmic antibodies* (ANCAs) are antibodies directed against neutrophil cytoplasmic antigens. Testing for ANCAs is important for the diagnosis and classification of various forms of vasculitis. In these disorders, the target antigens are proteinase 3 (PR3) and myeloperoxidase (MPO). Both antigens are located in the azurophilic granules of neutrophils and the peroxidase-positive lysosomes of monocytes. Antibodies that target PR3 and MPO are known as *PR3-ANCA* and *MPO-ANCA*.[8] There is an association between ANCA and several major vasculitic syndromes: granulomatosis with polyangiitis (GPA; formerly known as Wegener granulomatosis), microscopic polyangiitis, allergic granulomatosis with polyangiitis (AGPA; formerly known as Churg-Strauss syndrome), and certain drug-induced vasculitis syndromes.[8]

GPA is a vasculitis of unknown origin that can damage organs by restricting blood flow and destroying normal tissue. Although any organ system may be involved, the disorder primarily affects the respiratory tract (sinuses, nose, trachea, and lungs) and the kidneys. Approximately 90% of patients with active GPA have ANCAs. In patients with limited disease presentations, typically limited to the respiratory tract, up to 40% may be ANCA-negative. Thus, although a positive ANCA test is useful to support a suspected diagnosis, a negative ANCA test does not exclude it. For this reason, the ANCA test is usually not used alone to diagnose GPA.

In patients with vasculitis, immunofluorescence after ethanol fixation reveals two characteristic patterns: cytoplasmic (cANCA) and perinuclear (pANCA). With cANCA, there is diffuse staining throughout the cytoplasm, which is usually caused by antibodies against PR3. The pANCA pattern is characterized by staining around the nucleus and perinuclear fluorescence. In patients with vasculitis, the antibody causing this pattern is generally directed against MPO.

Although detection and identification of ANCAs is most useful in diagnosing various vasculitides, ANCAs have been reported in connective tissue diseases (e.g., RA, SLE, and myositis), chronic infections (e.g., cystic fibrosis, endocarditis, and HIV), and gastrointestinal diseases (e.g., inflammatory bowel disease, sclerosing cholangitis, and autoimmune hepatitis). Some medications may induce vasculitis associated with positive ANCA (usually MPO-ANCA).[9] The drugs most strongly associated with ANCA-associated vasculitis are propylthiouracil, methimazole, and carbimazole. Hydralazine, minocycline, penicillamine, allopurinol, procainamide, thiamazole, clozapine, phenytoin, rifampin, cefotaxime, isoniazid, and indomethacin are less commonly associated with the disorder.[8]

Quantitative Assay Results

When used in the diagnosis of GPA, the specificity of PR3-ANCA is approximately 90%. The sensitivity of the test is about 90% when the disease is active and 40% when the disease is in remission. Thus, the sensitivity of PR3-ANCA is related to the extent, severity, and activity of the disease at the time of testing.

The utility of obtaining serial PR3-ANCA tests in assessing disease activity is controversial. Some data suggest that a rise in titers predicts clinical exacerbations and justifies increasing immunosuppressive therapy. However, other studies have shown that disease flares cannot be predicted in a timely fashion by elevations in ANCA titers. Further, the immunosuppressive and cytotoxic therapies used are associated with substantial adverse effects. For these reasons, an elevation in ANCA should not be used as the sole justification for initiating immunosuppressive therapy. Rather, patients with rising ANCA titers should be monitored closely with therapy withheld unless there are clear clinical signs of active disease.[8]

Qualitative Assay Results

The sensitivity and specificity of cANCA, pANCA, and anti-MPO tests for various diseases are listed in **Table 19-2**. The presence of cANCAs denotes a spectrum of diseases ranging from idiopathic glomerulonephritis to extended GPA.[10] In most cases of vasculitis, renal disorder, and granulomatous disease, patient sera are negative for cANCAs.

The pANCA test has limited diagnostic value. A positive pANCA test should be followed by antigen-specific assays such as anti-MPO. In ulcerative colitis, the specificity of the pANCA test has been reported to be as high as 94%. However, with only moderate sensitivity and inconsistent correlation between titers and disease activity, pANCA screening may be of little value. Although sensitivity can reach 85% in primary sclerosing cholangitis, the pANCA test lacks specificity in the differential diagnosis of autoimmune hepatic diseases. In RA, pANCA may be related to aggressive, erosive disease. The sensitivity of the test increases in RA complicated by vasculitis, but its specificity remains low.

Complement

The *complement* system consists of at least 60 different plasma and membrane proteins that provide a defense mechanism against microbial invaders and serve as an adjunct or "complement" to humoral immunity. The system works by depositing complement components on pathologic targets and by the interaction of plasma proteins in a cascading sequence to mediate inflammatory effects such as opsonization of particles for phagocytosis, leukocyte activation, and assembly of the membrane attack complex (MAC).[11] Six plasma control proteins and five integral membrane control proteins regulate this cascade.

TABLE 19-2. Disease Associations of Antinuclear Cytoplasmic Antibodies (ANCAs)

ANCA	SENSITIVITY	SPECIFICITY
cANCA	Extended GPA: >90%	Systemic vasculitis or idiopathic rapidly progressive glomerulonephritis: 95%
	Limited GPA: 70–85%	
	Microscopic polyarteritis: 23%	
	Polyangiitis overlap syndrome: 40%	
	Idiopathic rapidly progressive glomerulonephritis: 30%	
	AGPA: 10%	
	Classic polyarteritis nodosa: 10%	
pANCA	Ulcerative colitis: 60–75%	Ulcerative colitis: >90%
	Crohn disease: 10–20%	Systemic vasculitis or idiopathic rapidly progressive glomerulonephritis: 81%
	Autoimmune chronic active hepatitis: 60–70%	
	Microscopic polyarteritis: 58%	
	Idiopathic crescentic glomerulonephritis: 45%	
	Primary biliary cirrhosis: 30–40%	
	Primary sclerosing cholangitis: 60–85%	
	RA with Felty syndrome: 90–100%	
	RA with vasculitis: 50–75%	
	RA: 20–40%	
Anti-MPO	Idiopathic rapidly progressive glomerulonephritis: 70%	Systemic vasculitis or idiopathic rapidly progressive glomerulonephritis: 91%
	AGPA: 70%	
	Microscopic polyarteritis: 50%	
	GPA: 20%	
	Classic polyarteritis nodosa: 20%	
	Polyangiitis overlap syndrome: 20%	

AGPA = allergic granulomatosis with polyangiitis; cANCA = cytoplasmic antineutrophil cytoplasmic antibody; GPA = granulomatosis with polyangiitis; MPO = myeloperoxidase; pANCA = perinuclear antineutrophil cytoplasmic antibody; RA = rheumatoid arthritis.

These proteins circulate normally in a precursor (inactive) form (e.g., C3 and C4). When the initial protein of a given pathway is activated, it activates the next protein (e.g., C3a and C4a) in a cascading fashion similar to that seen with coagulation factors.

Activation of this system can occur through any one of three proteolytic pathways:

1. **Classical pathway**—This pathway is activated when IgM or IgG antibodies bind to antigens such as viruses or bacteria.
2. **Alternative pathway**—This is an evolutionary surveillance system that does not require the presence of specific antibodies.
3. **Lectin pathway**—This pathway is activated similarly to the classical pathway, but instead of antibody binding, mannose-binding protein binds to sugar residues on the surface of pathogens.

Activation by any of the three pathways generates enzymes that cleave the third and fifth complement components (C3 and C5). A final common (or terminal) sequence culminates in the assembly of the MAC. Five proteins (C5 through C9) interact to form the MAC, which creates transmembrane channels or pores that displace lipid molecules and other elements, resulting in disruption of cell membranes and cell lysis.

Because the complement system is an important part of immune system regulation, complement deficiency predisposes an individual to infections and autoimmune syndromes. In disorders associated with autoantibodies and the formation of immune complexes, the complement system can contribute to tissue damage.

Serum complement levels reflect a balance between synthesis and catabolism. Hypocomplementemia occurs when the C3 or C4 concentration falls below its reference range. Most cases of hypocomplementemia are associated with hypercatabolism (complement depletion) due to activation of the immune system rather than decreased production of complement components (hyposynthesis). Most diseases associated with the formation of IgG- or IgM-containing circulating immune complexes can cause hypocomplementemia. Rheumatologic diseases included in this category are SLE, RA with extra-articular disease, Sjögren syndrome, and systemic vasculitis. Nonrheumatologic diseases associated with hypocomplementemia include antiphospholipid syndrome, subacute bacterial endocarditis, hepatitis B surface antigenemia, pneumococcal infection, gram-negative sepsis, viral infections (e.g., measles), recurrent parasitic infections (e.g., malaria), and mixed cryoglobulinemia.[11,12]

Because errors in interpretation of complement study results can occur, three important aspects should be considered when interpreting these results:

1. **Reference ranges are relatively wide.** Therefore, new test results should be compared with previous test results rather than with a reference range. It is most useful to examine serial test results and correlate changes with a patient's clinical picture.
2. **Normal results should be compared with previous results, if available.** Inflammatory states may increase the rate of synthesis and elevate serum complement protein levels. For example, some SLE patients have concentrations of specific complement components that are two to three times the upper limit of normal (ULN) when their disease is clinically inactive. When the disease activity increases to the point that increased catabolism of complement proteins occurs, levels may then fall into the reference range. It would be a misinterpretation to conclude that these "normal" concentrations represent an inactive complement system. Consequently, serial determinations of complement levels may be more informative than measurements at a single point.
3. **Complement responses do not correlate consistently with disease activity**. In some patients, the increase and decrease of the complement system should not be used to assess disease activity.

Assessment of the complement system should include measurement of the total hemolytic complement activity by the complement hemolytic 50% (CH_{50}) test and determination of the levels of C3 and C4.

Total Hemolytic Complement

Reference range: 150–250 IU/mL

The *total hemolytic complement* (THC or CH_{50}) measures the ability of a patient's serum to lyse 50% of a standard suspension of sheep erythrocytes coated with rabbit antibody. All nine components of the classical pathway are required to produce a normal reaction. The CH_{50} screening test may be useful when a complement deficiency is suspected or a body fluid other than serum is involved. For patients with SLE and lupus nephritis, serial monitoring of CH_{50} may be useful for guiding drug therapy.

C3 and C4

Reference ranges: C3, 72–156 mg/dL or 0.72–1.56 g/L; C4, 20–50 mg/dL or 0.2–0.5 g/L

Because C3 is the most abundant complement protein, it was the first to be purified and measured by immunoassay. However, C4 concentrations appear to be more sensitive to smaller changes in complement activation and more specific for identifying complement activation by the classic pathway. Results of C3 and C4 testing are helpful in following patients who initially present with low levels and then undergo treatment, such as those with SLE.

Acute-Phase Reactants

The concentration of a heterogeneous group of plasma proteins, called *acute-phase proteins* or *acute-phase reactants,* increases in response to inflammatory stimuli such as tissue injury and infection. Concentrations of CRP, serum amyloid A protein, α_1-acid glycoprotein, α_1-antitrypsin, fibrinogen, haptoglobin, prealbumin, ferritin, and complement characteristically increase, whereas serum transferrin, albumin, and transthyretin concentrations decrease. Their collective change is referred to as the *acute-phase response.*

In general, if the inflammatory stimulus is acute and of short duration, these proteins return to normal within days to weeks.

However, if tissue injury or infection is persistent, acute-phase changes may also persist. Additionally, white blood cell (WBC) and platelet counts may be elevated significantly.

Rheumatic diseases are chronic and associated with varying severities of inflammation. The ESR and CRP are two tests that can be helpful in three ways: (1) estimating the extent or severity of inflammation; (2) monitoring disease activity over time; and (3) assessing prognosis.[13] Unfortunately, both tests are nonspecific and cannot be used to confirm or exclude any particular diagnosis.

Erythrocyte Sedimentation Rate

Reference range (Westergren method): 0–15 mm/hr for males; 0–20 mm/hr for females

For many years, the *erythrocyte sedimentation rate* (ESR) has been used widely as a reflection of the acute-phase response and inflammation. The test is performed by placing anticoagulated blood in a vertical tube and measuring the rate of fall of erythrocytes in mm/hr. In rheumatologic diseases, the ESR is an indirect screen for elevated concentrations of acute-phase plasma proteins, especially fibrinogen.[13] An elevated ESR occurs when elevated protein concentrations (especially fibrinogen) cause aggregation of erythrocytes, resulting in a faster fall of those cells in the tube.

Several factors unrelated to inflammation may result in an increased ESR, such as obesity and increasing age. The ESR also responds slowly to an inflammatory stimulus. Despite these limitations, the test remains in wide use because it is inexpensive and easy to perform, and a tremendous amount of data are available about its clinical significance in numerous diseases. The Westergren method of performing an ESR test is preferred over the Wintrobe method because of the relative ease of performing the former method in clinical or laboratory settings.

Correlation of serial Westergren ESR results with patient data may influence therapeutic decisions. Two rheumatologic diseases, polymyalgia rheumatica and temporal arteritis (giant cell arteritis), are almost always associated with an elevated Westergren ESR. The ESR is usually >60 mm/hr and frequently >100 mm/hr in these disorders. During initial therapy or treatment initiated after a disease flare, a significant decrease or a return to a normal ESR usually indicates that systemic inflammation has decreased substantially. In the absence of clinical symptoms, an increased ESR may indicate that more aggressive therapy is needed. Disease activity can then be monitored by ESR results. Of course, if symptoms are present, they should not be ignored.

C-Reactive Protein

Reference range: 0–0.5 mg/dL or 0–0.005 g/L

C-reactive protein (CRP) is a plasma protein of the acute-phase response. In response to a stimulus such as injury or infection, CRP can increase up to 1000 times its baseline concentration. The precise physiologic function of CRP is unknown, but it is known to participate in activation of the classical complement pathway and interact with cells in the immune system.

Serum CRP levels can be quantitated accurately and inexpensively by immunoassay or laser nephelometry. Most healthy adults have concentrations of <0.3 mg/dL, although concentrations of 1 mg/dL are sometimes seen. Moderate increases range from 1–10 mg/dL, and marked increases are >10 mg/dL.[13] Values above 15–20 mg/dL are usually associated with bacterial infections. In general, concentrations >1 mg/dL reflect the presence of a significant inflammatory process. As with the Westergren ESR, serial measurements of CRP are the most valuable, especially in chronic inflammatory diseases.

Currently, the routine use of CRP for the assessment of rheumatologic diseases is limited. As with the ESR, CRP concentrations generally increase and decrease with worsening and improving signs and symptoms, respectively. Nevertheless, CRP concentrations are not specific for any disease.

Using an assay method called *high-sensitivity CRP (hs-CRP)*, several studies have shown a correlation between elevated levels and cardiovascular events including myocardial infarction. Although controversial, research suggests that CRP is simply a marker for atherosclerosis and cardiovascular disease rather than a cause.[14] The American College of Cardiology Foundation and American Heart Association (ACCF/ACC) 2010 guidelines for assessment of cardiovascular risk in asymptomatic adults provide recommendations for measurement of CRP in select patient populations.[15] In patients at intermediate risk of a cardiovascular event (i.e., Framingham 10-year CHD risk of 10–20%), an elevated CRP (>3 mg/L) is considered to confer high risk. A level of 1–3 mg/L is average risk, and a level <1 mg/L is low risk.[15] Levels >10 mg/L should be disregarded for coronary risk prediction purposes and the patient should be evaluated for clear sources of systemic inflammation or infection.

The units of measurement for the hs-CRP (mg/L) are different from those of the conventional CRP test (mg/dL). Because CRP levels fluctuate over time, when the hs-CRP is used for cardiovascular risk assessment, the test should be measured twice at least two weeks apart and the two values averaged.

Human Leukocyte Antigen B27 (HLA-B27)

Human leukocyte antigen B27 is an antigen on the surface of WBCs encoded by the B locus in the major histocompatibility complex on chromosome 6. The HLA-B27 test is qualitative and will be either present or absent. Its presence is associated with autoimmune diseases known as *seronegative spondyloarthropathies.* An HLA-B27 test may be ordered when a patient has pain and inflammation in the spine, neck, chest, eyes, or joints and an autoimmune disorder associated with the presence of HLA-B27 is the suspected cause. The test may be obtained to confirm a suspected diagnosis of ankylosing spondylitis, Reiter syndrome, or anterior uveitis. However, a positive test cannot distinguish among these diseases and cannot be used to predict progression, severity, prognosis, or the degree of organ involvement. Some patients with these disorders may have a negative HLA-B27 test. Further, the test cannot definitively diagnose or exclude any rheumatologic disease. It is frequently ordered in concert with other rheumatologic tests (e.g., RF, ESR, CRP), based on the clinical presentation.

A positive HLA-B27 in a person without symptoms or a family history of HLA-B27 associated disease is not clinically significant. For example, it does not help predict the likelihood of developing an autoimmune disease. The presence or absence of HLA antigens is genetically determined. If a family member has an HLA-B27 related rheumatologic disease, other family members who share the HLA-B27 antigen have a higher risk of developing a similar disease. Individuals who already know they are HLA-B27 positive may wish to seek genetic counseling to understand the hereditary impact on their family.

New genetic testing methods permit separation of HLA-B27 into subtypes. Approximately 15 subtypes have been identified; the most common are HLA-B27*05 and HLA-B27*02. The precise clinical significance of individual subtypes is an area of continuing investigation.

Synovial Fluid Analysis

Synovial fluid is essentially an ultrafiltrate of plasma to which synovial cells add hyaluronate. This fluid lubricates and nourishes the avascular articular cartilage. Normally, synovial fluid is present in small amounts and is clear and acellular (<200 cells/mm^3) with a high viscosity because of the hyaluronic acid concentration. Normal fluid does not clot because fibrinogen and clotting factors do not enter the joint space from the vascular space. Protein concentration is approximately one third that of plasma, and glucose concentration is similar to that of plasma.

When performing arthrocentesis (joint aspiration), a needle is introduced into the joint space of a diarthrodial joint. With a syringe, all easily removed synovial fluid is drained from the joint space. Arthrocentesis is indicated as a diagnostic procedure when septic arthritis, hemarthrosis (bleeding into a joint space), or crystal-induced arthritis is suspected. Furthermore, arthrocentesis may be indicated in any clinical situation, rheumatologic or nonrheumatologic, if the cause of new or increased joint inflammation is unknown. Arthrocentesis is also performed to administer intra-articular corticosteroids.

When arthrocentesis is performed, the synovium may be inflamed, allowing fibrinogen, clotting factors, and other proteins to diffuse into the joint. Therefore, the collected synovial fluid should be placed in heparinized tubes to prevent clotting and to allow determination of cell type and cell number. If diagnostic arthrocentesis is indicated, the aspirated joint fluid should be analyzed for volume, clarity, color, viscosity, cell count, culture, glucose, and protein. Synovial fluid is subsequently reported as normal, noninflammatory, inflammatory, or septic.[16] **Table 19-3** presents the characteristics of normal and three pathological types of synovial fluid. The presence and type of crystals in the fluid should be determined. The presence of crystals identified by polarized light microscopy with red compensation can be diagnostic (**Table 19-4**). (**Minicase 1**.)

TABLE 19-3. Synovial Fluid Characteristics and Classification

CHARACTERISTIC	NORMAL	NONINFLAMMATORY	INFLAMMATORY	SEPTIC
Viscosity	High	High	Low	Variable
Color	Colorless to straw	Straw to yellow	Yellow	Variable
Clarity	Transparent	Transparent	Translucent–opaque	Opaque
WBC (/mm^3)	<200	200–3000	3000–50,000	>50,000
PMN	<25%	<25%	Often >50%	>75%
Culture	Negative	Negative	Negative	Often positive
Glucose (a.m. fasting)	≅ blood	≅ blood	>25 mg/dL but lower than blood	<25 mg/dL (much lower than blood)
Protein (g/dL)	1–2	1–3	3–5	3–5

PMN = polymorphonuclear leukocyte; WBC = white blood cell; ≅ = approximately equal to.

TABLE 19-4. Morphology of Synovial Fluid Crystals Associated with Joint Disease

CRYSTALS	SIZE (MM)	MORPHOLOGY	BIREFRINGENCE[a]	DISEASES
Monosodium urate	2–10	Needles, rods	Negative	Gout
CPPD	2–10	Rhomboids, rods	Positive (weak)	CPPD crystal deposition disease (pseudogout), OA
Calcium oxalate	2–10	Polymorphic, dipyramidal shapes	Positive	Renal failure
Cholesterol	10–80	Rectangles with notched corners; needles	Negative or positive	Chronic rheumatoid or osteoarthritic effusions
Depot corticosteroids	4–15	Irregular rods, rhomboids	Negative or positive	Iatrogenic postinjection flare

CPPD = calcium pyrophosphate dihydrate; OA = osteoarthritis.

[a] The property of birefringence is the ability of crystals to pass light in a particular plane. When viewed under polarized light, the crystals are brightly visible in one plane (birefringent), but are dark in a plane turned 90 °. Birefringence observed under polarized light can be categorized as "positive" and "negative" based on the speed at which rays of light travel through the crystals in perpendicular planes (at right angles).

MINICASE 1

Assessment of Synovial Fluid

William D., a 79-year-old man, presents to his primary care physician with complaints of pain and swelling in his left knee after a busy day doing yardwork. His past medical history is significant for osteoarthritis. Physical examination reveals a swollen, inflamed, and painful left knee. He reports feeling fatigued and experiencing chills, and he has an oral temperature at home of 101 °F.

The left knee is aspirated and drained. Several drops of cloudy, yellow aspirate are placed on a slide and sent to pathology. The remaining aspirate is sent in heparinized tubes to the laboratory for Gram stain, bacterial culture, cell count, and chemistry panel. Examination of the slide reveals a mixture of rhomboid or rod shaped crystals. The slide is then viewed under a polarizing light microscope with a first-order, red plate compensator. Most crystals demonstrate weak positive birefringence.

After receiving the pathology report, the physician reviews the preliminary laboratory results of the knee aspirate (refer to Table 19-3 for reference values):

- 60,000 WBCs/mm^3
- 75% PMNs
- 23 mg/dL glucose
- 4.5 g/dL protein
- Gram-positive cocci were present on Gram stain

QUESTION: What is the likely diagnosis in this patient? What additional laboratory studies should be performed?

DISCUSSION: When he presents initially with acute monoarthritis, the most common differential diagnoses are crystal arthritis (i.e., gout, pseudogout), osteoarthritis, trauma, and infection. The presence of systemic signs and symptoms of infection creates high suspicion of septic arthritis. The aspiration of cloudy, yellow fluid from a red, swollen, and painful knee is consistent with infection and inflammation. Therefore, appropriate diagnostic tests were performed on the synovial fluid.

His history of osteoarthritis could suggest that joint overuse from doing yard work increased his arthritis symptoms. Microscopic examination revealed rhomboid and rod crystal shapes. Based on the weak positive birefringence findings, the crystals are probably composed of calcium pyrophosphate dihydrate, which may be present in a joint affected by osteoarthritis. However, the synovial fluid analysis also reveals elevated WBCs, PMNs, protein, low glucose, and the presence of Gram positive cocci (e.g., *Staphylococcus aureus*). Considering the patient's systemic findings of infection and the synovial fluid analysis, he requires aggressive medical treatment, including empiric antibiotics. Culture and sensitivity testing must be done to determine a specific antibiotic regimen.

Nonrheumatologic Tests

The three most commonly performed groups of nonrheumatologic tests performed in rheumatology are the *complete blood count* (CBC), *serum chemistry panel,* and *urinalysis.* These tests are not specific for any rheumatologic disorder, and abnormal results may occur in association with many rheumatologic and nonrheumatologic diseases. These tests are discussed from a more general perspective in other chapters.

The CBC includes hemoglobin, hematocrit, red blood cell (RBC) indices (MCV, MCH, and MCHC), WBC count, WBC differential, and platelet count. Chronic inflammatory diseases such as RA and SLE are commonly associated with anemia (low hemoglobin and hematocrit). The RBC indices often indicate that the anemia is normochromic and normocytic; this is often referred to as *anemia of chronic disease* or *anemia of inflammation*. Anemia may also be associated with a low MCV (microcytic anemia). Microcytic anemia accompanies chronic blood loss, which may occur as a result of drug therapy for rheumatologic diseases (e.g., gastroduodenal hemorrhage from nonsteroidal anti-inflammatory drugs [NSAIDs]). Additional tests may be necessary to rule out iron deficiency anemia (e.g., stool guaiac, serum iron, and total iron binding capacity). Autoimmune hemolytic anemia may be seen in SLE and other rheumatic diseases and is associated with a rapid onset that may be life threatening. It occurs as a result of formation of an IgG autoantibody to the RBC membrane. The Coombs test to detect the presence of antibodies is needed to diagnose hemolytic anemia. The platelet count may be elevated in some disorders (thrombocytosis) and decreased in others (thrombocytopenia). Leukopenia may be present, and the WBC differential may reflect either increases or decreases in various cell elements. Leukopenia may be associated with Felty syndrome and may also be caused by therapy with immunosuppressive agents used to treat rheumatologic diseases. For example, thrombocytopenia and leukopenia may be seen in SLE.

Antiphospholipid syndrome may be seen with SLE and other disorders and is associated with abnormalities in coagulation. It is diagnosed by detecting the presence of antiphospholipid antibodies on two separate occasions at least 12 weeks apart. Patients with antiphospholipid antibodies should be evaluated further to determine thrombosis risk.

The chemistry panel may include baseline measurements of electrolytes (e.g., sodium, potassium, chloride, and carbon dioxide) and tests of hepatic and renal function. SLE may be associated with hepatic dysfunction, which can be assessed by determination of hepatic transaminases (aspartate aminotransferase [AST], alanine aminotransferase [ALT]), total and direct bilirubin, alkaline phosphatase, and gamma glutamyl transferase. Some drugs used in the treatment of rheumatologic disease may also cause hepatic injury. Chronically poor nutrition may result in low serum albumin and total protein levels. Renal function tests (usually the serum creatinine (SCr) and blood urea nitrogen [BUN]) may provide evidence of renal involvement in patients with lupus nephritis. The urinalysis with microscopic evaluation is useful in detecting proteinuria, hematuria, and pyuria, which may be seen in SLE and with use of drugs to treat rheumatologic disorders.

INTERPRETATION OF LABORATORY TESTS IN SELECT RHEUMATOLOGIC DISEASES

Rheumatoid Arthritis in Adults

In 2010, the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) published new classification criteria for RA.[17] The criteria were developed to help define homogeneous treatment populations for research trials, not for clinical diagnosis. In practice, the clinician must establish the diagnosis for an individual patient using many more aspects (including perhaps some additional laboratory tests) based on the clinical presentation. Nevertheless, the formal classification criteria are useful as a general guide to making a clinical diagnosis. The 2010 criteria are aimed at diagnosing RA earlier in newly presenting patients so patients can be started on treatment sooner, with the goal of preventing erosive joint damage and improving long-term outcomes.[18] Patients with erosive disease typical of RA and those with longstanding disease (including patients whose disease is inactive with or without treatment) should also be classified as having RA if they previously fulfilled the 2010 criteria.[17]

Two mandatory criteria must be met before the classification criteria can be applied to an individual patient. First, there must be evidence of definite clinical synovitis in at least one joint as determined by an expert assessor; the distal interphalangeal joints, first carpometacarpal joints, and first metatarsophalangeal (MTP) joints are *excluded* from consideration because these joints are usually involved in osteoarthritis (OA). Second, the synovitis cannot be better explained by another disease such as SLE, psoriatic arthritis, or gout. When these two criteria are met, classification of definite RA is based on achieving a score of 6 or more out of 10 points in four domains:

1. Joint distribution
   - One large joint (shoulder, elbow, hip, knee, ankle) = 0 points
   - 2–10 large joints = 1 point
   - One to three small joints (metacarpophalangeal joints, proximal interphalangeal joints, 2nd–5th MTP, thumb interphalangeal joints, wrists) = 2 points
   - 4–10 small joints = 3 points
   - More than 10 joints (with at least one small joint) = 5 points
2. Serology
   - Negative RF and ACPA = 0 points
   - Low-positive RF or ACPA = 2 points
   - High-positive RF or ACPA = 3 points
3. Acute phase reactants
   - Normal CRP and ESR = 0 points
   - Elevated CRP or ESR = 1 point
4. Symptom duration (by patient report)
   - Less than six weeks = 0 points
   - Six weeks or more = 1 point

Patients who do not achieve a score of 6 or higher can be reassessed subsequently, because they may meet the classification criteria cumulatively over time.

Anticitrullinated Protein Antibodies

Patients with established RA are typically treated aggressively early in the disease course because most damage from bone erosions occurs within the first two years in 90% of patients. For this reason, an accurate early diagnosis is critical. Many patients with early RA have mild, nonspecific symptoms; in these cases, the ability to detect a disease-specific antibody such as ACPA could be of crucial diagnostic and therapeutic importance.

The ACPA test has several useful characteristics as a marker for RA diagnosis and prognosis: (1) it is as sensitive as RF and more specific than RF for RA in patients with early as well as fully established disease; (2) it may be detectable in seemingly healthy persons years before the onset of clinical RA findings; (3) it may predict the future development of RA in patients with undifferentiated arthritis; and (4) it may be a predictor for the eventual development of erosive disease. As stated previously, ACPAs can be detected in about 50–60% of patients with early RA, usually after having nonspecific symptoms for three to six months prior to seeing a physician.

In some patients with nonspecific arthritis, it is difficult to make a definitive diagnosis of RA because of the lack of disease-specific serum markers for other conditions in the differential diagnosis. In some situations, the presence of ACPAs may help differentiate RA from polymyalgia rheumatica or erosive forms of SLE.[3] However, the ACPA test is not 100% specific for RA; positive tests have also been reported in some patients with other autoimmune rheumatologic diseases (e.g., SLE, Sjögren syndrome, psoriatic arthritis), tuberculosis, and chronic lung disease.[19]

Several reports suggest that patients with early RA who are ACPA positive go on to develop more erosive disease than those who are antibody-negative.[3] Early identification of patients who are at risk for a more severe disease course could lead to more rapid and aggressive institution of disease-modifying therapies. However, clinical trials are needed to determine whether this diagnostic and therapeutic approach is indeed beneficial.

Rheumatoid Factor

In patients with RA, affected diarthrodial joints have an inflamed and proliferating synovium infiltrated with T lymphocytes and plasma cells. Plasma cells in the synovial fluid generate large amounts of IgG RF and abnormally low amounts of normal IgG. However, plasma cells in the bloodstream of patients with RA produce IgM RF predominantly.[2]

From 75–80% of adults with RA have a positive RF titer, and most of those who are positive have titers of at least 1:320. A positive RF is not specific for the diagnosis of RA. Some connective diseases, such as SLE and Sjögren syndrome, are also associated with positive RF titers. RF levels may also be increased in some infections (e.g., malaria, rubella, hepatitis C). Further, up to 5% of the normal healthy population may be RF positive. Patients with RA generally have higher RF titers than individuals with other nonrheumatologic conditions. In RA patients with a positive RF, the titer generally increases as disease activity (inflammation) increases. Consequently, as the serum RF titer increases, the specificity of the test for the diagnosis of RA also increases.[2] Higher titers or serum concentrations suggest the presence of more severe disease than with lower levels and are associated with a worse prognosis.

RF is one of the two serologic tests (along with ACPA) included in the 2010 ACR/EULAR classification criteria for RA.[17] Based on the reporting of RF levels in IU/mL, a *negative* RF is considered to be less than or equal to the ULN for the laboratory and assay. A *low-positive* test is higher than the ULN but less than or equal to three times the ULN. A *high-positive* test is more than three times the ULN for the assay. When the RF is reported as only positive or negative by the laboratory, patients reported as having a positive RF should be scored as "low-positive" for RF scoring purposes.[17]

Although RFs are usually identified and quantified from serum samples, RA is a systemic, extravascular, autoimmune disease affecting the synovium. As a result, some RFs may be present in sites other than peripheral blood. IgG RFs are found in the synovial fluid of many patients with severe RA. IgA RF may be detected in the saliva of patients with RA or Sjögren syndrome. The presence of IgE RF is correlated with extra-articular findings of RA.[2]

Although the majority of patients with RA are seropositive for RF, some patients have negative titers. However, some of these patients may have non-IgM RF, predominantly IgG RF. Also, some seronegative patients convert to seropositive on repeat testing. A small percentage of adult RA patients (<10%) are considered to be truly seronegative. When compared with RF-positive patients, seronegative patients usually have milder arthritis and are less likely to develop extra-articular manifestations (e.g., rheumatoid nodules, lung disease, and vasculitis).

Because current treatment guidelines call for aggressive early treatment of RA—before end-organ damage—clinicians must be aware of the relationship between disease onset and RF development. Unfortunately, the RF test is least likely to be positive at the onset of RA, when it might be of the most help. After RA has been diagnosed, RF titers are not routinely used to assess a patient's current clinical status or modify a therapeutic regimen. A specific titer or a change in titers for an individual does not correlate reliably with disease activity.

In summary, RF is not sensitive or specific enough to use as the sole laboratory test to diagnose or manage RA. Although it is present in the majority of patients with RA, it is negative in some patients with the disease. RF may be useful as a prognostic indicator, because RA patients with high RF titers generally have a more severe disease course.

Antinuclear Antibodies

ANAs are usually negative in patients with RA. The frequency of positive ANAs in patients with RA is highly variable. As determined by indirect immunofluorescence, this frequency varies from 10–70%, depending on the substrate used and the titer considered positive. In patients with a positive ANA, tests for dsDNA and Sm antibodies should be performed because these tests are highly specific for SLE.

Complement

The serum complement level is usually normal or elevated in RA. Complement elevations often occur as part of the acute-phase response. These increases parallel changes in other acute-phase proteins (e.g., CRP). Elevations of total hemolytic activity (CH_{50}), C3, and C4 are usually observed during active stages of most rheumatologic diseases, including RA. The presence of circulating immune complexes in RA may lead to hypercatabolism of complement and acquired hypocomplementemia.

Acute Phase Reactants

As in other inflammatory diseases, the nonspecific ESR test is usually elevated in active RA. The degree of elevation is directly related to the severity of inflammation; the Westergren ESR can be 50–80 mm/hr or more in patients with severely active RA. It usually decreases or normalizes when systemic inflammation decreases during initial treatment or after treatment for a disease flare. However, there is a large variability in response to treatment among individuals. Subsequent increases in disease activity will be mirrored by corresponding increases in ESR.

CRP levels may be elevated (approximately 2–3 mg/dL) in adult RA patients with moderate disease activity. However, there is substantial individual variability, and 5–10% of such patients have normal values. Some patients with severe disease activity have levels of 14 mg/dL or higher.

Because of their nonspecificity, the ESR and CRP are of little use in distinguishing between RA and other rheumatologic diseases such as OA or mild SLE. The tests also are elevated in patients with vasculitis associated with RA and reflect the generalized systemic inflammatory state. These tests are more appropriate for monitoring disease activity in RA. Elevations of ESR and CRP are individually associated with radiologic damage in RA as assessed by the number of joint erosions. Elevation of both ESR and CRP is a stronger predictor of radiologic progression than an elevation in CRP alone.[19,20]

Synovial Fluid Analysis

If a joint effusion is present and the diagnosis is uncertain arthrocentesis and *synovial fluid analysis* are performed to exclude gout, pseudogout, or infectious arthritis.[19] Synovial fluid analysis should include WBC count with differential, analysis for crystals, and Gram stain and culture. In early RA, the analysis typically reveals straw-colored, turbid fluid with fibrin fragments.[15] A clot will form if the fluid is left standing at room temperature. There are usually 5000–25,000 WBC/mm^3, at least 85% of which are PMNs. Complement C4 and C2 levels are usually slightly decreased, but the C3 level is generally normal. The glucose level is decreased, sometimes to <25 mg/dL. No crystals should be present, and cultures should be negative.

Nonrheumatologic Tests

The CBC may reveal an anemia that is either normochromic-normocytic (anemia of chronic disease) or hypochromic-microcytic (MCV <80 μm^3). Anemia of chronic disease is not associated with erythropoietin deficiency. Microcytic anemia is due to iron deficiency that may result from gastrointestinal blood loss associated with drug use (e.g., NSAIDs) or other causes. Further testing must be performed to identify the source of bleeding (e.g., stool guaiac testing and endoscopy). The WBC count may show a slight leukocytosis with a normal differential. Eosinophilia (>5% of the total WBC count) may be associated with RF-positive severe RA. Felty syndrome may be associated with granulocytopenia.

Thrombocytosis may be present in clinically active RA as part of the acute-phase response. As the disease improves spontaneously or as a result of drug therapy, the platelet count returns toward normal.

Serum chemistries may reveal low serum albumin and total protein levels because of poor nutrition and loss of appetite. Renal function, hepatic injury tests, and urinalysis should be normal. Abnormalities in renal or liver function caused by comorbid conditions are important because they may affect choice of pharmacotherapy or drug dosing.

Juvenile Idiopathic Arthritis

The term *juvenile idiopathic arthritis* (JIA) encompasses a heterogeneous group of childhood arthritis conditions of unknown cause. Because JIA includes a variety of arthritis categories that differ from adult-onset RA, the term JIA has replaced the name *juvenile rheumatoid arthritis*.[21] Juvenile idiopathic arthritis begins before the 16th birthday and persists for at least six weeks; other known causes must be excluded before the diagnosis of JIA can be made. Juvenile idiopathic arthritis is divided into categories based on presenting clinical and laboratory findings: (1) systemic arthritis; (2) oligoarthritis; (3) polyarthritis (RF negative); (4) polyarthritis (RF positive); (5) psoriatic arthritis; (6) enthesitis-related arthritis; and (7) undifferentiated arthritis.[21,22]

The diagnosis of JIA is made primarily on clinical grounds. There is no single laboratory test or combination of tests that can confirm the diagnosis. However, laboratory tests can be useful in providing evidence of inflammation, supporting the clinical diagnosis, and monitoring toxicity from therapy. (**Minicase 2**.)

Rheumatoid Factor

RF-positive polyarthritis constitutes 5–10% of JIA cases. It is defined as arthritis affecting five or more joints in the first six months of disease with a positive RF test on two occasions at least three months apart.[23] RF-positive polyarthritis is 6–12 times more common in girls than boys. As in adult RA, the RF test usually detects IgM-anti-IgG. RF-negative polyarthritis constitutes 20–30% of new JIA cases. It also includes arthritis in five or more joints during the first six months, but the RF test is negative.

Oligoarthritis is the most common form of JIA; it is four times more common in girls than boys and has a peak onset

MINICASE 2

Evaluation of Arthritis

Joan G., a 62-year-old postmenopausal woman, presents to her family physician complaining of pain and stiffness in her hands for the last month. She notes the pain is worse in the morning when she wakes up and resolves about two hours later. Her past medical history is significant for hypertension and dyslipidemia for approximately five years. She quit smoking one year ago following a 20 pack-per-year smoking history. She started a diet (low fat, low sodium) and exercise (walking 30 minutes daily) program about two months ago. On physical exam, symmetrical tenderness and swelling are present in her MCPs and PIPs. Current medications (stable for the past six months) include lisinopril, hydrochlorothiazide, atorvastatin, and one aspirin tablet a day. She is allergic to sulfa and intolerant to penicillin (stomach upset). She started taking ibuprofen about one month ago in response to the joint pain.

Laboratory results obtained at this visit:

- RF titer 1:60
- ACPA 60 EU/mL
- ANA 1:40
- ESR 50 mm/hr
- CRP 1 mg/dL

QUESTIONS: What are two likely diagnoses, which one is most likely, and what data support that diagnosis?

DISCUSSION: The two most common causes of joint pain and stiffness include OA and RA. Upon initial presentation, either may be a possibility. The physical exam findings are key to identifying joints that are typically affected by RA versus OA. Morning stiffness is a common symptom of either condition; however, morning stiffness associated with RA typically lasts for more than an hour, whereas stiffness associated with OA lasts 30 minutes or less. The laboratory results are also helpful in distinguishing between the two conditions. First, OA does not involve systemic inflammation, so it is unlikely to cause elevated acute phase reactants (ESR and CRP). Second, RF is negative; however, the presence of positive RF is not specific for a diagnosis of RA. Finally, ACPA is sensitive and specific for RA, and the results are positive. According to the ACR classification criteria for RA, this patient has more than 10 joints involved (3 points), high-positive ACPA (3 points), elevated CRP and ESR (1 point), with a duration of less than six weeks (0 points). Therefore, this total score of 7 meets the classification criteria for definite RA (6 or greater).

before the age of six years. Oligoarthritis affects four or fewer joints in the first six months; the RF test is usually negative. The RF is negative in systemic arthritis, psoriatic arthritis, enthesitis-related arthritis, and undifferentiated arthritis.[23]

Anticitrullinated Protein Antibodies

The APCA test alone is not helpful in diagnosing a subcategory of JIA. This is consistent with the fact that JIA is a heterogeneous group of disorders, most of which are different from adult RA. Similar to RA in adults, positive ACPA in JIA have been associated with RF-positive disease and erosive arthritis.[23] Approximately 60–70% of patients with RF-positive polyarthritis are ACPA positive. Approximately 50–80% of patients with RF-negative polyarthritis are ACPA positive.[23]

Antinuclear Antibodies

In oligoarthritis, 50–70% of children have positive ANA tests, typically 1:40 to 1:320. The highest prevalence of ANA seropositivity (65% to 85%) is seen in young girls with oligoarticular onset JIA and uveitis. The ANA test is positive in 40% of patients with RF-negative polyarthritis and positive in up to 55% of patients with RF-positive polyarthritis. The ANA is positive in about 15–20% of children with psoriatic arthritis. It may be positive in some patients with enthesitis-related arthritis. The test is seldom positive (<10%) in children with systemic JIA.

Complement

As with adult-onset RA, serum complement components (especially C3) are usually elevated in systemic JIA.

Acute Phase Reactants

In systemic arthritis, the ESR and CRP are typically very high during an acute flare. In oligoarthritis, there is little systemic inflammation, and the ESR and CRP are usually normal. Some cases of oligoarthritis may be associated with mildly or moderately elevated ESR or CRP; however, elevated acute phase reactants in this category should raise suspicion for other conditions, such as subclinical inflammatory bowel disease associated with arthropathy. Acute phase reactants may be elevated in either RF-positive or RF-negative polyarthritis and in psoriatic arthritis. The ESR may be elevated in enthesitis-related arthritis, but this abnormality should raise suspicion for subclinical inflammatory bowel disease. Acute phase reactants are typically mildly elevated in patients with the psoriatic arthritis subcategory of JIA.

Synovial Fluid Analysis

Arthrocentesis in JIA is typically consistent with inflammatory fluid. As in adult RA, synovial fluid glucose levels are low.

Nonrheumatologic Tests

Children with systemic arthritis may have anemia, leukocytosis with neutrophilia, and thrombocytosis. The anemia is normochromic-normocytic (anemia of chronic disease); hemoglobin values may be in the range of 7–10 g/dL. WBC counts in the range of 20,000–30,000 cells/mm^3 are not uncommon, and counts may exceed 60,000–80,000 cells/mm^3. In severe cases, liver enzymes, ferritin, and coagulation screen also may be abnormal. Patients with enthesitis-related or psoriatic arthritis may have a mild anemia of chronic disease.

Systemic Lupus Erythematosus

Criteria for the classification of *systemic lupus erythematosus* (SLE) were developed by the ACR in 1971 and revised in 1982 and 1997 (**Table 19-5**).[24,25] The criteria were developed for research purposes to ensure that SLE patients reported in the literature actually have the disease.[26] The wide variety of manifestations and unpredictable course often make SLE difficult to diagnose. The classification criteria can be helpful in establishing the diagnosis, especially for patients with longstanding, established disease. Because SLE tends to involve organ systems sequentially over a period of years, the classification system lacks sensitivity for patients early in the disease course.

The diagnosis of SLE may be made if any four of the 11 classification criteria are present, serially or simultaneously, during any observation period. However, even if four criteria are fulfilled a clinician may elect not to diagnose SLE because of contradictory history and physical examination findings. Likewise, patients with only three classification criteria may have their signs and symptoms diagnosed as SLE when strong clinical suspicion is present.[27]

In 2012, the Systemic Lupus International Collaborating Clinics released a classification criteria famework for SLE that differs from the 1997 ACR criteria.[26] The intent of the 2012 framework is to improve some of the weaknesses of the 1997 criteria. Although the sensitivity improved, the updated framework may delay diagnosis or miss diagnoses. Clinicians are encouraged to use both documents to support clinical judgment during initial evaluation and diagnosis.[28]

Antinuclear Antibodies

ANA testing is usually performed initially if SLE is suspected because of its high sensitivity and ease of use. At least 95% of active, untreated patients with SLE have a positive ANA, usually at a titer of 1:160 or higher.[28] For patients presenting with rheumatologic signs and symptoms (e.g., joint pain, joint swelling, and morning stiffness) and signs suggestive of SLE (e.g., butterfly rash, photosensitivity, oral ulcers, and discoid rash), a positive ANA test is one of the 11 possible SLE classification criteria established by the ACR (Table 19-5).[24,25] On the other hand, a negative ANA test does not exclude the diagnosis in patients with typical features of the disease.

The ANA test has low specificity for SLE; many other conditions are associated with a positive test (e.g., systemic sclerosis, polymyositis, dermatomyositis, RA, autoimmune thyroiditis or hepatitis, infections, malignancies, and many drugs). Some healthy persons also may have a positive ANA test. Consequently, results of an ANA test are always interpreted in light of a patient's clinical presentation.

Anti-dsDNA and Anti-ssDNA Antibodies

The *antidouble-stranded DNA* (anti-dsDNA) test is positive in 70% of patients with SLE at some point in the disease course, and

TABLE 19-5. ACR Revised Classification Criteria for SLE[a]

CRITERION	DEFINITION
1. Malar rash	Fixed erythema, flat or raised, over the malar eminences, tending to spare the nasolabial folds
2. Discoid rash	Erythematous raised patches with adherent keratotic scaling and follicular plugging; atrophic scarring may occur in older lesions
3. Photosensitivity	Skin rash as a result of unusual reaction to sunlight, by patient history or physician observation
4. Oral ulcers	Oral or nasopharyngeal ulceration, usually painless, observed by a physician
5. Arthritis	Nonerosive arthritis involving two or more peripheral joints, characterized by tenderness, swelling, or effusion
6. Serositis	a) Pleuritis—convincing history of pleuritic pain or rub heard by a physician or evidence of pleural effusion
	OR
	b) Pericarditis—documented by electrocardiogram or rub or with evidence of pericardial effusion
7. Renal disorder	a) Persistent proteinuria >0.5 g/day or >3+ if quantitation not performed OR
	b) Cellular casts—may be red cell, hemoglobin, granular, tubular, or mixed
8. Neurologic disorder	a) Seizures—in the absence of offending drugs or known metabolic derangements (e.g., uremia, ketoacidosis, or electrolyte imbalance)
	OR
	b) Psychosis—in the absence of offending drugs or known metabolic derangements (e.g., uremia, ketoacidosis, or electrolyte imbalance)
9. Hematologic disorder	a) Hemolytic anemia—with reticulocytosis
	OR
	b) Leukopenia—<4000/mm^3 total on two or more occasions
	OR
	c) Lymphopenia—<1500/mm^3 on two or more occasions
	OR
	d) Thrombocytopenia—<100,000/mm^3 in the absence of offending drugs
10. Immunologic disorder	a) Anti-DNA: antibody to native DNA in abnormal titer
	OR
	b) Anti-Sm: presence of antibody to Sm nuclear antigen
	OR
	c) Positive finding of antiphospholipid antibodies based on (1) an abnormal serum level of IgG or IgM anticardiolipin antibodies, (2) a positive test result for lupus anticoagulant using a standard method, or (3) a false-positive serologic test for syphilis known to be positive for at least 6 months and confirmed by Treponema pallidum immobilization or fluorescent treponemal antibody absorption test
11. ANA	Abnormal titer of ANA by immunofluorescence or an equivalent assay at any point in time and in the absence of drugs known to be associated with drug-induced lupus syndrome

ANA = antinuclear antibody; DNA = deoxyribonucleic acid; IgG = immunoglobulin G; IgM = immunoglobulin M.

[a] For the purpose of identifying patients in clinical studies, a person shall be said to have SLE if any four or more of the 11 criteria are present, serially or simultaneously, during any interval of observation.

Source: Adapted from references 24 and 25.

the test is 95% specific for SLE. In contrast, testing for antibodies to single-stranded DNA (anti-ssDNA) has poor specificity for SLE but is more sensitive (90%). Although the anti-ssDNA antibody appears to be important in the immunopathogenesis of SLE, the test has little diagnostic utility because of poor specificity. There is evidence that anti-dsDNA and anti-ssDNA antibodies are important in the pathogenesis of lupus nephritis because they appear to correlate with its presence and severity. Titers of these antibodies tend to fall with successful treatment, frequently becoming undetectable during sustained remission.

Anti-Sm, Anti-Ro/SSA, and Anti-La/SSB Antibodies

The *anti-Sm* antibody is an immunoglobulin specific against Sm, a ribonucleoprotein found in the cell nucleus. The anti-Sm antibody test is positive in 10–30% of SLE patients, and presence of these antibodies is pathognomonic for SLE.[27] *Anti-Ro/SSA* and *anti-La/SSB* antibodies are present in 10–50% and 10–20% of patients with SLE, respectively, but they are not specific for the disease.

Complement

Total CH_{50} levels are decreased at some point in most patients with SLE. Complement levels decrease in SLE because of deposition of immune complexes in active disease (hypercatabolism). Complement depletion has been associated with increased disease severity, particularly renal disease. Analysis of various complement components has revealed low levels of C1, C4, C2, and C3. Serial determinations have demonstrated that decreased levels may precede clinical exacerbations.[11] As acute episodes subside, levels return toward normal. Some authorities consider it helpful to follow complement measurements in SLE patients receiving treatment, especially if C4 and C3 were low at the time of diagnosis.[11]

Acute Phase Reactants

Serum ESR and CRP concentrations are elevated in many patients with active SLE, but many individuals have normal CRP levels. Those with acute serositis or chronic synovitis are most likely to have markedly elevated CRP levels. Patients with other findings of SLE, such as lupus nephritis, may have modest or no elevations.

Several studies have examined the hypothesis that elevations in CRP during the course of SLE result from superimposed infection rather than activation of SLE. In hospitalized patients, substantially elevated serum CRP levels occur most frequently in the setting of bacterial infection. Consequently, CRP elevations >6–8 mg/dL in patients with SLE (as well as other diseases) should signal the need to exclude the possibility of infection. Such CRP increases should not be considered proof of infection, because CRP elevation can be related to active SLE in the absence of infection.

Nonrheumatologic Tests

Antiphosopholipid antibodies (i.e., anticardiolipin antibodies and the lupus anticoagulant) can occur as an idiopathic disorder and in patients with autoimmune and connective tissue diseases such as SLE.[29] Anticardiolipin antibody and the lupus anticoagulant are closely related but are different antibodies. Consequently, an individual can have one antibody and not the other. These antibodies react with proteins in the blood that are bound to phospholipid, a type of fat molecule that is part of normal cell membranes. Antiphospholipid antibodies interfere with the normal function of blood vessels by causing narrowing and irregularity of the vessel (vasculopathy), thrombocytopenia, and thrombosis. These changes can lead to complications such as recurrent deep venous thrombosis, stroke, myocardial infarction, and fetal loss. The presence of these antibodies may increase the risk of future thrombotic events. This clinical situation is referred to as the *antiphospholipid syndrome* (APS). The diagnosis of APS is made when an individual has an antiphospholipid antibody documented either by a solid-phase assay (anticardiolipin) or by a test for an inhibitor of phospholipid-dependent clotting (lupus anticoagulant) along with a clinical event.[29] When APS occurs in patients with no other diagnosis, it is referred to as *primary APS*. Patients who also have SLE or another rheumatologic disease are said to have secondary APS.

Anemia is present in many patients with SLE. The CBC may reveal a normochromic-normocytic anemia (anemia of chronic disease) that is not associated with erythropoietin deficiency. Hemolytic anemia with a compensatory reticulocytosis also may occur due to antierythrocyte antibodies. This is one of 11 diagnostic criteria for SLE (Table 19-5) and occurs in approximately 10% of patients. The majority of patients also have a positive Coombs test. Anemia in SLE also can result from blood loss, renal insufficiency, medications, infection, hypersplenism, and other reasons.[27]

Leukopenia, also one of the SLE classification criteria, is common but usually mild. It results primarily from decreased numbers of lymphocytes, which may be caused by the disease or its treatment. If the patient is not being treated with corticosteroids or immunosuppressive agents, ongoing immunologic activity should be suspected. Neutropenia in SLE may occur from immune mechanisms, medications, bone marrow suppression, or hypersplenism.[27]

Mild thrombocytopenia (100,000–150,000/mm^3) occurs in 25–50% of patients with SLE and is usually due to immune-mediated platelet destruction. Increased platelet consumption and impaired platelet production also may be contributing factors.[27] Also, liver function tests may reveal increased hepatic aminotransferases (AST, ALT), lactate dehydrogenase, and alkaline phosphatase in patients with active SLE. These elevations usually decrease as the disease improves with treatment. The urinalysis with microscopic analysis may show proteinuria (>500 mg/24 hr) in about 50% of patients. Hematuria and pyuria also may occur. However, renal disease may exist in the presence of a normal urinalysis.

Osteoarthritis

Osteoarthritis (OA) results from the complex interplay of numerous factors, such as joint integrity, genetics, mechanical forces, local inflammation, and biochemical processes.[30] It is not generally considered to be an autoimmune disease. The synovium is normal, and the synovial fluid usually lacks inflammatory cells. Although affected joints are painful, they are frequently not inflamed. The primary use of laboratory tests when OA is suspected is to rule out other disorders in the differential diagnosis.

There are no clinical laboratory tests that are specific for the diagnosis of OA. Laboratory tests that may be performed in patients suspected of having OA include ESR, RF titers, and evaluation of synovial fluid.[30] The ESR (and CRP) is usually normal, but may be slightly increased if inflammation is present. The RF test is negative, and serum chemistries, hematology tests, and urinalysis are normal.

Synovial fluid analysis may be undertaken, especially in patients with severe, acute joint pain. Findings generally reveal either a noninflammatory process or mild inflammation (WBC <2000 cells/mm^3). Crystals are absent when the synovial fluid is examined using compensated polarized light microscopy.

Fibromyalgia

Fibromyalgia is a common syndrome associated with pain, fatigue, sleep disturbances, and other medical problems.[31] It is a challenging syndrome to categorize and treat. According to the criteria for fibromyalgia established by an ACR committee in 1990, an individual must have both a history of chronic widespread pain and tenderness at 11 or more of 18 specific tender point sites on physical examination.[32] According to the less restrictive preliminary criteria established by ACR in 2010, a patient must have a history of widespread pain and multiple symptoms (e.g., muscle pain and tenderness, somatic symptoms, unrefreshed sleep).[32] The 2012 preliminary criteria must undergo validation before they are considered a replacement for the 1990 criteria. However, many people who carry the clinical diagnosis of fibromyalgia do not meet these precise criteria. In fact, some authorities contend that a formal diagnosis of fibromyalgia is unnecessary provided that fibromyalgia symptoms are recognized.[30]

Laboratory testing should be used prudently when evaluating patients with clinical features suggestive of fibromyalgia. A satisfactory patient assessment is usually obtained by a careful medical history and physical examination and perhaps performance of routine laboratory tests, such as CBC and serum chemistry to rule out other disorders. Serologic tests such as ANA titers are not usually necessary unless there is strong evidence of an autoimmune disorder.

If the results of laboratory testing suggest a diagnosis other than fibromyalgia, a more directed evaluation is required. Individuals who actually have fibromyalgia are sometimes misdiagnosed with autoimmune disorders. This may be due to the common complaints of arthralgias, myalgias, fatigue, morning joint stiffness, and a history of swelling of the hands and feet. Conversely, patients with existing autoimmune diseases may suffer from symptoms suggestive of fibromyalgia.

TESTS TO MONITOR DRUG THERAPY FOR SELECT RHEUMATOLOGIC DISORDERS

Pharmacotherapy for rheumatologic diseases can cause significant adverse reactions that are reflected in laboratory test results.[34-36] Abnormal test results may necessitate dose reduction, temporary discontinuation, or permanent withdrawal of the offending drug. The laboratory tests most commonly affected are the WBC count, platelet count, hepatic aminotransferases, total bilirubin, SCr, BUN, and urinalysis (**Table 19-6**).

A decrease in WBC count (leukopenia) is considered to be clinically significant when the total count is 3000–3500 cells/mm^3 (3–3.5 × 10^9/L) or lower, because immune defenses are compromised. Leukopenia is due primarily to a relative and absolute decrease of neutrophils (neutropenia). Pancytopenia indicates a suppression of all cell lines, including WBCs, RBCs, (anemia), and platelets (thrombocytopenia). Aplastic anemia indicates a complete arrest of blood cell production in the bone marrow. Increases in hepatic aminotransferases of two to three times baseline may be clinically important. In the urinalysis, the most common abnormalities identified are proteinuria, hematuria, pyuria, and casts.

TESTS TO GUIDE MANAGEMENT OF GOUT AND HYPERURICEMIA

The serum uric acid and urine uric acid concentrations are the two most commonly used tests to diagnose gout and assess the effectiveness of its treatment. The BUN and SCr also should be monitored as appropriate.

Serum Uric Acid

Reference range: 4–8.5 mg/dL (237–506 μmol/L) for males >17 years old; 2.7–7.3 mg/dL (161–434 μmol/L) for females >17 years old

Uric acid is the metabolic end-product of the purine bases of DNA. In humans, uric acid is not metabolized further and is eliminated unchanged by renal excretion. It is completely filtered at the renal glomerulus and is almost completely reabsorbed. Most excreted uric acid (80–86%) is the result of active tubular secretion at the distal end of the proximal convoluted tubule.[37]

As urine becomes more alkaline, more uric acid is excreted because the percentage of ionized uric acid molecules increases. Conversely, reabsorption of uric acid within the proximal tubule is enhanced and uric acid excretion is suppressed as urine becomes more acidic.

In plasma at normal body temperature, the physicochemical saturation concentration for urate is 7 mg/dL. However, plasma can become supersaturated, with the concentration exceeding 12 mg/dL. In nongouty subjects with normal renal function, urine uric acid excretion abruptly increases when the *serum uric acid* concentration approaches or exceeds 11 mg/dL. At this concentration, urine uric acid excretion usually exceeds 1000 mg/24 hr.

Hyperuricemia

When serum uric acid exceeds the upper limit of the reference range, the biochemical diagnosis of *hyperuricemia* can be made. Hyperuricemia can result from an overproduction of purines and reduced renal clearance of uric acid. When specific factors affecting the normal disposition of uric acid cannot be identified, the problem is diagnosed as *primary* hyperuricemia. When specific factors can be identified (e.g., another disease or drug therapy), the problem is referred to as *secondary* hyperuricemia.

As the serum urate concentration increases above the upper limit of the reference range, the risk of developing clinical signs and symptoms of gouty arthritis, renal stones, uric acid nephropathy, and subcutaneous tophaceous deposits increases. However, many hyperuricemic patients are asymptomatic. If a patient is hyperuricemic, it is important to determine if there are potential causes of false laboratory test elevation and contributing extrinsic factors. In general, clinical studies have not shown that impaired renal function is caused by chronic hyperuricemia (unless there are other renal risk factors and excluding acute uric acid nephropathy resulting from tumor

TABLE 19-6. Routine Laboratory Tests to Monitor Patients Receiving Select Drugs for Treatment of Rheumatoid Arthritis, Systemic Lupus Erythematosus, or Osteoarthritis

DRUG	DISEASE	LABORATORY TEST	ADVERSE DRUG REACTION	INCIDENCE (%)
Abatacept	RA	Baseline tuberculin skin test	Infection, sepsis	Rare
		CBC with differential and platelet count		
		Monitor for infection		
Anakinra	RA	CBC with differential and platelet count	Neutropenia	8
		Monitor for infection	Infection, sepsis	Rare
Hydroxychloroquine	RA	CBC with differential and platelet count	Thrombocytopenia	0–6
		Urinalysis	Proteinuria	0–6
Leflunomide	RA	CBC with differential and platelet count	Pancytopenia	<1
		Hepatic aminotransferases, bilirubin	Elevated aminotransferases	5–10
			Hepatic necrosis	Rare
Methotrexate	RA	CBC with differential and platelet count	Leukopenia	0–3
		Hepatic aminotransferases, bilirubin, serum albumin	Pancytopenia Hepatotoxicity	0–2 4–21
		Monitor for infection	Infection, sepsis	Rare
Rituximab	RA	CBC with differential and platelet count	Infection, sepsis	Rare
		Monitor for infection		
Sulfasalazine	RA	CBC with differential and platelet count	Leukopenia	0–3
		Hepatic aminotransferases	Hepatotoxicity	1–6
Tacrolimus	RA	BUN, SCr, tacrolimus trough blood concentrations	Nephrotoxicity	Variable
		Serum potassium, magnesium, phosphorus	Hyperkalemia, hypomagnesemia, hypophosphatemia	Variable
		Hepatic aminotransferases, bilirubin	Hepatotoxicity	Variable
TNF antagonists (adalimumab, certolizumab, etanercept, golimumab, infliximab)	RA	Baseline tuberculin skin test	Activation of tuberculosis	Rare
		CBC with differential and platelet count	Infection, sepsis	Rare
		Monitor for infection		
Tocilizumab	RA	CBC with differential and platelet count	Infection, sepsis	Rare
		Monitor for infection	Hepatic enzyme elevations	Rare
		Hepatic transaminases		
		Fasting lipid panel	Dyslipidemia	Variable
Tofacitinib	RA	Baseline tuberculin skin test	Infection, sepsis	Rare
		CBC with differential and platelet count	Hepatic enzyme elevations	Rare
		Monitor for infection		
		Fasting lipid panel	Dyslipidemia	Variable
Belimumab	SLE	Monitor for infection	Infection, sepsis	Variable
Mycophenolate mofetil	SLE	CBC with differential and platelet count	Neutropenia, red cell aplasia	23–45
		Monitor for infection	Opportunistic infections, sepsis	Variable
Corticosteroids	RA, OA, SLE	CBC with differential and platelet count	Anemia due to peptic ulceration and blood loss	Rare
		Serum sodium, potassium, bicarbonate	Electrolyte disturbances	Variable
		Serum calcium	Osteoporosis	Variable
		Blood glucose	Hyperglycemia	Variable
		Fasting lipid panel	Dyslipidemia	Variable
		Urinalysis	Glycosuria	Variable

(continued)

TABLE 19-6. Routine Laboratory Tests to Monitor Patients Receiving Select Drugs for Treatment of Rheumatoid Arthritis, Systemic Lupus Erythematosus, or Osteoarthritis, cont'd

DRUG	DISEASE	LABORATORY TEST	ADVERSE DRUG REACTION	INCIDENCE (%)
NSAIDs, including aspirin	RA, OA, SLE	CBC with differential and platelet count	Anemia (due to gastroduodenal ulceration and blood loss)	Variable
		Hepatic aminotransferases	Hepatotoxicity	Rare
		BUN, SCr; sodium, potassium	Nephrotoxicity; electrolyte disturbances	Rare
		Urinalysis	Proteinuria, hematuria, pyuria	Rare
Allopurinol	Gout and hyperuricemia	CBC with differential and platelet count	Myelosuppression	Rare
		Serum uric acid	Gout flare (monitor uric acid)	Variable
		Hepatic aminotransferases	Hepatotoxicity	Rare
		BUN, SCr	Nephrotoxicity	Rare
Febuxostat	Gout and hyperuricemia	Serum uric acid	Gout flare (monitor uric acid)	Variable
		Hepatic aminotransferases	Elevated aminotransferases	5–7
Colchicine	Gout and hyperuricemia	CBC with differential and platelet count	Myelosuppression	Rare
		Hepatic aminotransferases	Elevated aminotransferases	Rare
		BUN, SCr		
Pegloticase	Gout and hyperuricemia	Serum uric acid	Gout flare (monitor uric acid)	74
Probenecid	Gout and hyperuricemia	Serum uric acid	Gout flare (monitor uric acid)	Variable
		BUN, SCr		

BUN = blood urea nitrogen; CBC = complete blood count; NSAIDs = nonsteroidal anti-inflammatory drugs; OA = osteoarthritis; RA = rheumatoid arthritis; SLE = systemic lupus erythematosus; TNF = tumor necrosis factor.
Source: Adapted from references 34–38.

lysis syndrome). However, long-term, very high serum uric acid levels (e.g., ≥13 mg/dL in men and 10 mg/dL in women) may predispose individuals to renal dysfunction. This level of hyperuricemia is uncommon, and a conclusive link to renal insufficiency has not been established. Also, renal disease accompanying hyperuricemia is often related to uncontrolled hypertension. Correction of hyperuricemia has no measurable effect on renal function.[38]

Exogenous causes. Medications are the most common exogenous causes of hyperuricemia. The two primary mechanisms whereby drugs increase serum uric acid concentrations are (1) decreased renal excretion resulting from drug-induced renal dysfunction or competition with uric acid for secretion within the kidney tubules, and (2) rapid destruction of large numbers of cells from antineoplastic therapy for leukemias and lymphomas.

The reduction in glomerular filtration rate accompanying renal impairment decreases the filtered load of uric acid and causes hyperuricemia. A number of drugs cause hyperuricemia by renal mechanisms that may include interference with renal clearance of uric acid. These agents include low-dose aspirin, pyrazinamide, nicotinic acid, ethambutol, ethanol, cyclosporine, acetazolamide, hydralazine, ethacrynic acid, furosemide, and thiazide diuretics. Diuretic-induced volume depletion results in enhanced tubular reabsorption of uric acid and a decreased filtered load of uric acid. Salicylates, including aspirin, taken in low doses (1–2 g/day) may decrease urate renal excretion. Moderate doses (2–3 g/day) usually do not alter urate excretion. Large doses (>3 g/day) generally increase urate renal excretion, thereby lowering serum urate concentrations.

Many cancer chemotherapeutic agents (e.g., methotrexate, nitrogen mustards, vincristine, 6-mercaptopurine, and azathioprine) increase the turnover rate of nucleic acids and the production of uric acid. Drug-induced hyperuricemia after cancer chemotherapy, especially high-dose regimens, can lead to acute renal failure. Allopurinol is routinely administered prophylactically to decrease uric acid formation. In other clinical situations, drug-induced hyperuricemia may not be clinically significant.

The decision to continue or discontinue a drug that may be causing hyperuricemia is dependent on three factors: (1) the risk of precipitating gouty symptoms, based on the patient's past history and current clinical status; (2) the feasibility of substituting another drug that is less likely to affect uric acid disposition; and (3) the plausibility of temporarily or permanently discontinuing the drug. If the regimen of the causative drug must remain unchanged, pharmacologic treatment of hyperuricemia may be instituted.

Diet is another exogenous cause of hyperuricemia. High-protein, weight-reduction programs can greatly increase the amount of ingested purines and subsequent uric acid production. If the average daily diet contains a high proportion of meats, the excess nucleoprotein intake can lead to increased uric acid production. Fasting or starvation also can cause hyperuricemia because of increased muscle catabolism. Furthermore, lead poisoning from paint, batteries, or "moonshine," in addition to recent alcohol ingestion, obesity, diabetes mellitus, and hypertriglyceridemia, are associated with increases in serum uric acid concentration. (**Minicase 3**.)

Endogenous causes. Endogenous causes of hyperuricemia include diseases, abnormal physiological conditions that may or may not be disease related, and genetic abnormalities. Diseases include (1) renal diseases (e.g., renal failure); (2) disorders associated with increased destruction of nucleoproteins (e.g., leukemia, lymphoma, polycythemia, hemolytic anemia, sickle cell anemia, toxemia of pregnancy, and psoriasis); and (3) endocrine abnormalities (e.g., hypothyroidism, hypoparathyroidism, pseudohypoparathyroidism, nephrogenic diabetes insipidus, and Addison disease).

Predisposing abnormal physiological conditions include shock, hypoxia, lactic acidosis, diabetic ketoacidosis, alcoholic ketosis, and strenuous muscular exercise. In addition, males and females are at risk of developing asymptomatic hyperuricemia at puberty and menopause, respectively. Genetic abnormalities include Lesch-Nyhan syndrome, gout with partial absence of the enzyme hypoxanthine guanine phosphoribosyltransferase, increased phosphoribosyl pyrophosphate P-ribose-PP synthetase, and glycogen storage disease type I.

Hypouricemia

Hypouricemia is not important pathophysiologically, but it may be associated with low-protein diets, renal tubular defects, xanthine oxidase deficiency, and drugs (e.g., high-dose aspirin, allopurinol, probenecid, and megadose vitamin C).

Assays and Interferences with Serum Uric Acid Measurements

In the laboratory, the concentration of uric acid is measured by either the phosphotungstate colorimetric method or the more specific uricase method. With the colorimetric

MINICASE 3

Hyperuricemia and Gout

Matthew B., a 45-year-old obese man, began a daily exercise program two weeks ago in an attempt to lose 50 lb. In addition, he has begun a high-protein liquid diet because he knows that "fatty foods are not healthy." He sees his family physician for his first complete physical examination in approximately seven years. He tells his physician he has recently started walking briskly for one hour three times a week and is watching his diet carefully. The only abnormal finding on physical examination is a BP of 150/95 mm Hg. After drawing blood for a CBC with differential and a full chemistry panel and obtaining a urine sample for urinalysis, the physician prescribes hydrochlorothiazide 25 mg once daily for hypertension. Three days later, he is notified that his laboratory results, including serum uric acid, are normal.

Two weeks later, he returns on crutches to see his physician. He explains that he injured his right foot three days prior when taking his daily walk before sunrise, and he accidentally stubbed his right foot on a rock. He also says he woke up two days prior with a fever and felt as if his right great toe was "in a vise while an ice-cold knife was being pushed into the joint."

Examination of his right foot reveals abrasions on all five toes. The skin of the great toe appears shiny, the toe is swollen and warm to the touch, and he is in obvious pain. Whitish fluid oozes from a small wound on the dorsal aspect of the great toe. After anesthetizing the joint, the physician aspirates several drops of the whitish fluid. The physician then performs a Gram stain and examines the fluid on a slide, finding needle-shaped crystals but no bacteria. He also orders a serum uric acid level.

QUESTION: What is this patient's likely diagnosis and prognosis?

DISCUSSION: He probably has experienced his first acute gout attack. Although his previous serum uric acid concentration was described as normal, one endogenous and two exogenous factors may have precipitated this attack. Hypertension is frequently associated with hyperuricemia. Also, the sudden change to a high-protein diet greatly increased his ingestion of purines, which are metabolized to uric acid. Finally, he also was started on hydrochlorothiazide, which is an inhibitor of the renal clearance of uric acid. The abrupt change in physical exertion probably did not contribute to the attack because it was low in intensity.

Although he attributes the condition to his traumatic toe-stubbing event, his physician notes that none of his other abraded toes appear to be "infected." Examination of the synovial fluid using a polarizing-light microscope reveals monosodium urate crystals without bacteria (Table 19-4). An elevated serum uric acid level would be consistent with a diagnosis of gout, but some patients can have acute gout attacks with a serum urate level that is within the reference range.

With appropriate treatment for acute gout, discontinuation of hydrochlorothiazide, and adequate follow-up monitoring, the patient's symptoms should improve substantially within 24–48 hours. Some patients never have a second gout attack, whereas others experience frequent and severe episodes. If the serum uric acid concentration that was ordered is reported as highly elevated (>10 mg/dL), initiating therapy to reduce hyperuricemia may be considered after resolution of the acute attack. According to the 2102 ACR guidelines for treating hyperuricemia, patients should be evaluated on a case-by-case basis to determine causes for elevated uric acid and need for urate-lowering therapy.[40] Any patient with frequent attacks (defined as two or more per year), tophi, chronic kidney disease stage 2 or greater, or a history of urolithiasis should receive urate-lowering therapy.[39] The results of a 24-hour urine collection would be useful in determining whether the patient is an overproducer or underexcretor of uric acid and help to guide therapy with either allopurinol or probenecid, respectively.

method, ascorbic acid, caffeine, theophylline, levodopa, propylthiouracil, and methyldopa can all falsely elevate uric acid concentrations. With the uricase method, purines and total bilirubin >10 mg/dL can cause a false depression of uric acid concentrations. False elevations may occur if ascorbic acid concentrations exceed 5 mg/dL or if plasma hemoglobin exceeds 300 mg/dL (in hemolysis).

Urine Uric Acid Concentration

Reference range: 250–750 mg/24 hr (1.48–4.46 mmol/24 hr)

In hyperuricemic individuals who excrete an abnormal amount of uric acid in the urine (hyperuricaciduria), the risk of uric acid and calcium oxalate nephrolithiasis increases. However, the prevalence of stone formation is only twice that observed in the normouricemic population. When a stone does form, it rarely produces serious complications. Furthermore, treatment can reverse stone disease related to hyperuricemia and hyperuricaciduria.

Pathologically, uric acid nephropathy—a form of acute renal failure—is a direct result of uric acid precipitation in the lumen of collecting ducts and ureters. Uric acid nephropathy most commonly occurs in two clinical situations: (1) patients with marked overproduction of uric acid secondary to chemotherapy-induced tumor lysis (leukemia or lymphoma), and (2) patients with gout and profound hyperuricaciduria. Uric acid nephropathy also has developed after strenuous exercise or convulsions.[37]

In hyperuricemia unrelated to increased uric acid production, quantification of urine uric acid excreted in 24 hours can help to direct prophylaxis or treatment. Patients at higher risk of developing renal calculi or uric acid nephropathy (patients with gout or malignancies) excrete 1100 mg or more of uric acid per 24 hours. Prophylaxis may be recommended for these patients; allopurinol should be used instead of uricosuric agents (e.g., probenecid) to minimize the risk of nephrolithiasis. Prophylactic therapy may be started at the onset of gouty symptoms.[37]

SUMMARY

Diagnosing and managing rheumatologic diseases rely heavily on a thorough medical history and physical exam. Clinicians can use laboratory tests to help confirm or rule out specific diagnoses. Every laboratory test ordered must be carefully evaluated to determine the next steps in an individual patient's care.

When used alone, no single test is diagnostic for any particular disease. However, positive RF and positive ANA tests are commonly observed in patients with RA and SLE, respectively.

The cANCA antibody is highly specific for the disease spectrum of GPA, and anti-MPO antibodies are highly specific for systemic vasculitis and idiopathic crescentic glomerulonephritis. The most complete screen of complement activation includes measurements of C3, C4, and CH_{50}. The Westergren ESR and CRP tests are nonspecific markers of systemic inflammation and must be interpreted in light of the clinical presentation and other laboratory tests.

Patients with hyperuricemia are usually asymptomatic. After treatment of an episode of acute gout, the decision to initiate antihyperuricemic therapy depends upon the frequency and severity of acute attacks. Allopurinol or febuxostat therapy is recommended for patients at risk of forming renal calculi.

LEARNING POINTS

1. ***How are laboratory tests used in patients with RA compared to those with SLE?***

   ANSWER: Most specific rheumatologic laboratory tests are used in the diagnosis or management of patients with RA or SLE. Positive results of RF testing are most commonly seen in patients with RA. Although higher concentrations of RF are associated with more severe disease, RF titers or concentrations are not used to assess disease severity or clinical response to treatment. ANA testing is most frequently performed in SLE diagnosis. A positive ANA occurs in the majority of patients diagnosed with drug-induced lupus or MCTD. Antidouble-stranded DNA and anti-Sm are disease-specific for SLE. The degree of general systemic inflammation can be estimated with the Westergren ESR and CRP tests. In RA, polymyalgia rheumatica, and temporal arteritis, elevated ESRs may indicate the need for more aggressive drug therapy. Unlike the ESR, CRP does not appear to increase with age and may be useful in assessing potential infection in SLE patients. RA may be associated with anemia of chronic disease and thrombocytosis, and SLE is normally associated with anemia of chronic disease and thrombocytopenia and occasionally with hemolytic anemia. Proteinuria, hematuria, and pyuria are often seen on urinalysis in SLE patients with active disease. When RA or SLE patients begin drug therapy, laboratory tests must be performed regularly to monitor for adverse drug effects.

2. ***How important are the sensitivity and specificity of laboratory tests for diagnosing or assessing rheumatologic diseases?***

   ANSWER: Sensitivity is defined as the ability of a test to show positive results in patients who actually have the disease. If a test is 100% sensitive, all patients with the disease will have a positive result. Low sensitivity leads to a high rate of false-negative results. For example, if a new test to diagnose RA is only 75% sensitive, this means that 25% of patients who actually have the disease will show a negative result. Specificity is defined as the ability of a test to show negative results in patients who do not actually have the disease. If a test is 100% specific, all patients who test positive actually have the disease. Lack of specificity leads to a high rate of false-positive results. If a new test to diagnose RA is only 75% specific, 25% of people tested who have a positive result do not actually have RA. For some laboratory tests, poor specificity is due to substances not associated with the disease that cross-react with the target compound.

3. ***What issues should be considered before ordering a laboratory test for a patient with a rheumatologic disorder?***

   ANSWER: Laboratory testing can be expensive and may be inconvenient to perform. Consequently, a number of questions should be posed before ordering another test. First, are the results of other tests already available that provide the same information? If a test was performed previously, are there important reasons to repeat the test now? Has enough time elapsed since the previous test to make new results meaningful? Will the results of this test change the diagnosis, prognosis, or therapeutic interventions I might make? In other words, will knowing this result change what I do? Are the benefits to the patient worth the possible discomfort, inconvenience, and extra cost? The results of laboratory tests should always be interpreted in light of the clinical picture (i.e., the patient's signs and symptoms).

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20

CANCERS AND TUMOR MARKERS

Patrick J. Medina and Sarah A. Schmidt

OBJECTIVES

After completing this chapter, the reader should be able to

- **Define tumor markers, describe the characteristics of an ideal tumor marker, and discuss the usefulness of tumor markers in the diagnosis, staging, and treatment of malignant diseases**
- **List malignant and nonmalignant conditions that may increase carcinoembryonic antigen levels and define the role of carcinoembryonic antigen in the management of colon cancer**
- **Describe how CA-125 may be used to diagnose and monitor ovarian cancer**
- **Describe how human chorionic gonadotropin and α-fetoprotein are used to diagnose and monitor germ cell tumors**
- **Discuss the role of estrogen and progesterone receptors and human epidermal growth factor receptor 2 in determining treatment decisions for breast cancer**
- **Outline the role of the *BCR-ABL* gene in the diagnosis and as a target for treatment in patients with chronic myelogenous leukemia**
- **Describe how mutations in epidermal growth factor receptor, V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog, v-Raf murine sarcoma viral oncogene homolog B1, or anaplastic lymphoma kinase are used in determining treatment decisions for melanoma, lung cancer, and colorectal cancer**

For most types of cancer, treatment is likely to be most successful if the diagnosis is made while the tumor mass is relatively small. Unfortunately, many common types of cancer (e.g., carcinomas of the lung, breast, and colon) are frequently not diagnosed until the tumor burden is relatively large and the patient has developed symptoms related to the disease. As the search for more effective treatments for cancer has intensified, much effort and many resources also have been dedicated to elucidating new methods of detecting cancers earlier while the tumor burden is low and the patient is asymptomatic. These efforts have led to improved radiologic and other diagnostic imaging, and the identification of biologic substances, which occur in relation to the tumor and can be detected even at very low concentrations in the blood or other body fluids.

The term *tumor marker* is used to describe a wide range of proteins that are associated with various malignancies. Typically, these markers are either proteins that are produced by or in response to a specific type of tumor, or they may be other physiologic proteins that are produced by malignant cells in excess of the normal concentrations. In either case, the concentration of the marker usually correlates with the volume of tumor cells (e.g., as the tumor grows or the number of malignant cells increases, the concentration of the marker also increases). In other cases the presence of a biologic marker may be used to predict response to treatment (e.g., the estrogen receptor [ER] or progesterone receptor [PR] in breast cancer) or to monitor the effects of treatment. More recently, some tumor markers have been shown to be essential to the viability of tumor cells, and specific therapies have been developed that target these markers of disease. These tumor markers are often identified by genetic mutations, translocations, or amplification of genetic material.

This chapter describes tumor markers that are used clinically to detect cancers, monitor cancer burden, and help choose drug therapy as well as the laboratory methods used to measure them. In addition the sensitivity, specificity, and factors that may interfere with evaluation of these tests are briefly discussed. For tumor markers that are widely used to screen for cancers, to confirm a cancer diagnosis, or to assess response to treatment, the clinical applications are described.

TUMOR MARKERS

Tumor markers may be found in the blood or other body fluids or may be measured directly in tumor tissues or lymph nodes. They can be grouped into three broad categories: (1) tumor-specific proteins are markers that are produced only by tumor cells—these proteins usually occur as a result of translocation of an oncogene and may contribute to the proliferation of the tumor; (2) nonspecific proteins related to the malignant cells including proteins that are expressed only during embryonic development and by cancer cells; and (3) proteins that are normally found in the body but are expressed or secreted at a much higher rate by malignant cells than normal cells.[1] In addition to the laboratory tests that are described in this chapter, it also should be remembered that abnormalities in other commonly used laboratory tests may provide some evidence that a malignancy exists. However, they are not related to specific tumors. For example, suppression of blood counts may represent infiltration of the bone marrow by tumor cells. Increased uric acid

TABLE 20-1. Serum Tumor Markers in Clinical Use

TUMOR MARKER	MALIGNANT DISEASE	SCREENING	DIAGNOSIS	STAGING OR PROGNOSIS	MONITORING TREATMENT OUTCOME OR DISEASE RECURRENCE	COMMENTS
PSA	Prostate carcinoma	X		X	X	Usually combined with digital rectal examination of the prostate for screening Inflammatory disorders of the prostate, instrumentation of the genitourinary tract, and mechanical manipulation of the prostate by biopsy, transurethral resection of the prostate or prostatectomy may increase PSA Certain medications may decrease PSA including 5-α reductase inhibitors, NSAIDs, statins, and thiazide diuretics Herbal products (e.g., saw palmetto) may also decrease PSA
CEA	Colon and breast carcinoma			X (in colon)	X	Hepatic cirrhosis, hepatitis, pancreatitis, peptic ulcer disease, hypothyroidism, ulcerative colitis, or Crohn disease may elevate CEA
CA 15-3, CA 27.29	Breast carcinoma			X	X Metastatic disease only	Other cancers (e.g., gastric, colorectal, lung), benign breast disease, and liver disease may all elevate levels
CA-125	Ovarian carcinoma			X	X	Endometriosis, ovarian cysts, liver disease, or pregnancy may elevate CA-125; in certain high-risk groups (strong family history) CA-125 in combination with ultrasound technology may be used to screen asymptomatic patients
hCG	Germ cell tumors of ovaries and testes; hydatidiform mole		X	X	X	Pregnancy, other types of cancer, or marijuana use may elevate hCG
CA 19-9	Pancreatic carcinoma			X	X	Pancreatitis, cirrhosis, gastric, and colon cancer may elevate CA 19-9
AFP	Hepatocellular carcinoma Testicular (nonseminoma-tous germ cell tumors)	X (hepato-cellular carcinoma)	X	X	X	Pregnancy; hepatitis; cirrhosis; and pancreatic, gastric, lung, and colon cancers all can elevate AFP; some non-U.S. countries, which have a high incidence of hepatocellular cancer, use AFP to screen for hepatocellular cancer
B_2M	Multiple (plasma cell) myeloma Chronic lymphocytic leukemia			X	X	Lymphomas, chronic lymphocytic leukemia, and renal failure may elevate

AFP = α-fetoprotein; B_2M = β-2 microglobulin; CEA = carcinoembryonic antigen; hCG = human chorionic gonadotropin; NSAIDs = nonsteroidal anti-inflammatory drugs; PSA = prostate specific antigen.

and lactate dehydrogenase are frequently associated with large tumor burdens. Alkaline phosphatase is frequently elevated in patients with tumors of the biliary tract or bone. Occasionally, tumors may also produce hormones in excessive amounts, such as calcitonin or adrenocorticotropin.

Clinical Uses

Tumor markers are used for several purposes including detection of occult cancers in asymptomatic individuals (e.g., cancer screening and early detection), determining the relative extent or volume of disease (staging), estimating prognosis, predicting and assessing responsiveness to treatment, and monitoring for disease recurrence or progression.[1] **Table 20-1** lists many of the commonly used tumor markers found in blood and their clinical applications. **Table 20-2** lists tumor markers found on tumor cells or genetic abnormalities found in tumor cells and their clinical applications. **Table 20-3** lists genetic mutations or translocations that help determine the best therapy. The characteristics of an ideal tumor marker are somewhat dependent on the specific application. Normal values are provided although laboratory reference ranges (normal values) may slightly differ, as will the interpretation of the laboratory value in an individual patient. For example, rising levels of a tumor marker that are still in the normal range may indicate early tumor recurrence.

Sensitivity and Specificity

For a tumor marker to be clinically useful, it must have a high degree of *sensitivity* and *specificity*. That is, the presence of the marker should correlate with the presence of the tumor, and

TABLE 20-2. Tumor Markers Found on Tumor Cells in Clinical Use

TUMOR MARKER	MALIGNANT DISEASE	SCREENING	DIAGNOSIS	STAGING OR PROGNOSIS	MONITORING TREATMENT (OUTCOME OR DISEASE RECURRENCE)	COMMENTS
ER/PR	Breast carcinoma			X		Used to determine benefit of hormonal therapies
HER2	Breast carcinoma			X		Used to determine benefit of anti-HER2 therapies
BCR-ABL gene	CML		X		X	Can be elevated in acute lymphoblastic leukemia and rarely in acute myeloid leukemia

CML = chronic myeloid leukemia; ER = estrogen receptor; HER2 = human epidermal growth factor receptor 2; PR = progesterone receptor.

TABLE 20-3. Tumor Markers Found on Tumor Cells in Clinical Use to Individualize Treatment

TUMOR MARKER	MALIGNANT DISEASE	TEST OUTCOME OF INTEREST	IMPACT ON DRUG SELECTION
EGFR	Lung	Mutation in exon 19 or 21	Mutation predictive of response to EGFR tyrosine kinase inhibitors
KRas	Colorectal cancer	Mutation versus wild type (nonmutated)	EGFR monoclonal antibodies (cetuximab and panitumumab) only effective against wild type
BRAF	Melanoma	BRAF V600E and V600K mutation	BRAF mutation predicts response to vemurafenib, dabrafenib, and trametinib
ALK	Lung cancer	ALK rearrangement	ALK rearrangement predicts response to ALK-inhibitors (crizotinib, ceritinib)

ALK = anaplastic lymphoma kinase; BRAF = v-Raf murine sarcoma viral oncogene homolog B1; EGFR = epidermal growth factor receptor; KRas = V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog.

a negative test should indicate, with some certainty, that the patient does not have the cancer. Chapter 1 describes the methodology in determining sensitivity and specificity and should be reviewed prior to reading this chapter. Knowledge of the sensitivity, specificity, and predictive values of tumor marker tests are particularly important when they are used to screen asymptomatic patients. If the tumor marker test is positive only in a portion of the patients that actually have the cancer or if the test is negative in patients who do have the disease, then diagnoses would be missed. In the case of malignant diseases, delay of the diagnosis until symptoms or other clinical findings appear may mean the difference between curable and incurable disease.

Outcomes studies evaluating the usefulness of tumor marker testing in asymptomatic individuals must result in decreased mortality rates due to the disease, not just establishment of a diagnosis. On the other hand, false-positive tests not only cause a high level of anxiety, they also typically result in the performance of very costly and sometimes invasive, additional diagnostic tests. Because of these limitations, the only tumor marker routinely used to screen for malignancies is prostate specific antigen (PSA). Although this is the single example, the use of PSA alone for prostate cancer screening is declining because it does not appear to reduce mortality.[2]

Sensitivity and specificity are also important when tumor marker tests are used to monitor for recurrent disease in patients who have previously been treated for the cancer. A negative tumor marker test that is known to have a high degree of specificity will give the patient, their family, and their clinicians a great deal of comfort and sense of security that the disease has been eliminated. If the test has a lower degree of sensitivity, then it is likely that other screening and diagnostic tests will need to be performed at regular intervals to monitor for disease recurrence. In some cases, the presence of a positive tumor marker may be indication enough to resume cancer treatment. A decision to initiate or resume treatment should be made when there is a high degree of certainty that there is actual disease present because most cancer treatments are associated with significant toxicity and a small, but appreciable, mortality risk. When tumor markers are used to assess the extent of disease or the presence of specific tumor characteristics (e.g., HER2/neu), the quantitative sensitivity may also be important in determining prognosis, appropriate diagnostic tests, and treatment options. Genetic mutational testing is a dichotomous endpoint that is present or not present; however, depending on the quality of tissue and type of testing, the sensitivity (false negatives) can be affected.

Accessibility

If a tumor marker test is to be used to screen asymptomatic individuals for cancer, both the individuals and their clinicians are more likely to include them if they do not necessitate painful, risky, or lengthy procedures to obtain the necessary fluid or tissue. Most clinicians request and patients willingly provide samples of blood, urine, or sputum in the course of regular physical examinations. However, if a test requires biopsy of other tissues or involves procedures that are associated with a significant risk of morbidity, patients and clinicians are likely only to consent to or include them in physical examinations if there is a high likelihood—or other evidence that supports the presence—of the disease. Tumor markers that are obtained from tumor tissue directly are obtained at the time of diagnosis with the original tissue.

Cost-Effectiveness

Widespread screening of asymptomatic individuals with a tumor marker test can be quite expensive. It is not surprising that insurance companies, health plans, and health policy decision-makers are also more likely to support the inclusion of these tests during routine physical examinations or other screening programs if health economic evaluations demonstrate that they may result in lower overall treatment costs and a positive benefit to society, such as prolongation of the patient's productivity.

Prostate Specific Antigen

Standard reference range: 0–4 ng/mL (4 mcg/L)

Prostate specific antigen (PSA) is a protein produced by both normal (benign) and malignant that is secreted into the blood. The role of PSA in the screening, diagnosis, and monitoring of treatment response of patients with prostate cancer is reviewed in Chapter 23.

Carcinoembryonic Antigen

Normal range: <2.5 ng/mL nonsmokers (<2.5 mcg/L); <5 ng/mL smokers (5 mcg/L)

Carcinoembryonic antigen (CEA) is a protein that is found in fetal intestine, pancreas, and liver. In healthy adults, the level of this protein is usually <2.5 ng/mL. Serum CEA levels are frequently elevated in patients with colon, breast, gastric, thyroid, or pancreatic carcinomas and a variety of nonmalignant conditions including hepatic cirrhosis, hepatitis, pancreatitis, peptic ulcer disease, hypothyroidism, ulcerative colitis, and Crohn disease. Occasionally, CEA also is elevated in patients with lung cancer. CEA levels are usually modestly increased in individuals who smoke, and the normal serum level in these individuals is usually considered to be <5 ng/mL. Nonmalignant conditions are usually not associated with CEA levels >10 ng/mL. However, many patients with malignant conditions will have CEA levels that greatly exceed 10 ng/mL.

Blood samples for CEA testing preferably should be obtained in a red top tube. Following separation of the serum (or plasma), the specimen can be refrigerated if it is to be assayed within 24 hours or frozen at –20 °C if the specimen is to be assayed later. Immunoassays from different manufacturers may provide different values; therefore, the same laboratory and assay method should be used whenever possible for repeat testing in an individual patient.

CEA is most commonly used in the assessment of colon cancer. Unfortunately, this test does not have adequate sensitivity or specificity to make it a useful screening test for asymptomatic individuals. It may be elevated in a wide variety of conditions as noted above and may be negative in patients with widely metastatic disease. It is most commonly used in monitoring patients with a known history of colon cancer.[2] Following detection of early stage colon cancer by screening tests and further diagnostic workup, a baseline serum CEA level is usually measured to determine if the tumor produces excessive amounts of CEA. If the CEA level is grossly increased, then the CEA level may be used to monitor the success of treatment or for evidence of tumor recurrence following successful treatment.

The CEA level also may provide some information on a patient's prognosis.[3,4] The elevation of CEA level may relate to the extent of disease (stage), which often correlates with overall survival. Following surgical removal of a colon cancer, the CEA level should return to normal (<2.5 ng/mL) within four to six weeks.[5] If the CEA level remains elevated beyond this point, it may indicate that either residual primary tumor or metastatic disease is present.

The CEA level should decline to below the 5 ng/mL level within one month following surgery if all of the tumor was successfully removed.[5] If the CEA remains elevated, there is a high likelihood that the tumor will recur, and many surgeons would even consider a second-look surgery at that time for identification and removal of residual disease. In early stage colon cancer (stages II and III), CEA levels should be followed every three to six months for five years after the completion of all cycles of adjuvant chemotherapy.[6] Rising CEA levels mandate evaluation of the patient for metastatic disease. In patients with metastatic disease CEA levels should be monitored at the start of therapy and then every one to three months during therapy.[6] Rising levels may indicate therapy failure, although increasing levels may result from chemotherapy at the beginning of treatment and require careful evaluation.[6,7] When CEA levels are monitored in conjunction with other follow-up tests including CT scans of the liver and colonoscopy, several studies have reported improved overall survival and other benefits, including cost-effectiveness, that are attributable to earlier detection of recurrent disease.[6,8]

CEA may also be used to monitor breast cancer patients with metastatic disease. The American Society of Clinical Oncology guidelines for use of tumor markers in breast cancer state that CEA levels in combination with imaging, medical history, and physical exam may indicate treatment failure and prompt evaluation for worsening of disease.[9] Rising CEA levels alone should not be used to monitor treatment efficacy. Unlike colon cancer, monitoring of CEA levels in early stage breast cancer (stages I to III) is not recommended after a patient has received primary therapy. (**Minicase 1**.)

CA 15-3 Antigen

Normal range: <30 units/mL

Cancer antigen 15-3 (CA 15-3) is defined by an assay using monoclonal antibodies directed against circulating mucin antigen shed from human breast cancer. In addition to elevation in the serum of many women with breast cancer, it may

MINICASE 1

A Case of Elevated CEA Levels

Phil L., a 64-year-old white male, presents to the clinic with a six-week history of worsening diarrhea (five to six stools a day), pain in his right upper quadrant, and general gastrointestinal discomfort. Additional past medical history includes hypercholesterolemia for the past five years. Medications include simvastatin 20 mg daily. He drinks one to two glasses of wine a day and has a 30 pack/year history of smoking.

A review of systems reveals lethargy and slight confusion but no apparent distress. Vital signs show a sitting BP of 125/75 mm Hg (standing BP not measured), a regular HR of 86 beats/min, and a rapid and shallow RR of 36 breaths/min. His physical examination is pertinent for signs of dehydration (poor skin turgor). Laboratory values are drawn. They are unremarkable except for serum sodium 153 mEq/L, serum creatinine 1.7 mg/dL, and BUN 45 mg/dL. The decision is made to admit him based on his dehydration and worsening diarrhea. Additional laboratory values are drawn in the hospital, including a CEA level of 27 ng/mL.

QUESTION: What is the most likely cause of this patient's fluid status? How is the CEA level interpreted in relation to colon cancer? Should any other laboratory or imaging tests be obtained to further assess if he has a malignant tumor?

DISCUSSION: This patient most likely has a malignant tumor in his colon. Common signs and symptoms of colon cancer include pain and a change in bowel habits, which result from the tumor blocking part of the colonic lumen and interfering with normal colonic function. This can lead to the severe diarrhea and dehydration as seen in this patient.

Although other nonmalignant conditions and smoking also are associated with increased CEA levels, levels >10 ng/mL indicate a high likelihood of cancer. An elevated CEA level alone is not enough to make a diagnosis of colon cancer and a complete workup including CT scans and a tissue diagnosis will need to be obtained prior to therapy. Additional laboratory values that may be useful would be CA 19-9 levels and a complete hepatic panel to assess for metastatic disease.

The CEA level may be used in him to monitor the success of treatment, to check for evidence of tumor recurrence following primary treatment, and to provide some indication of his prognosis. Following successful surgery the CEA level should return to normal (<2.5 ng/mL) within four to six weeks. Depending on the stage of disease, Phil L. will have his CEA levels followed periodically to assess for disease recurrence or progression of metastatic disease.

also be elevated in lung cancer and other nonmalignant conditions including liver and breast disorders. Elevated CA 15-3 has been demonstrated to be a poor prognostic factor in early stage breast cancer, but the test is not sensitive enough to use as a screening test for early stage breast cancer.[10] This test is used in combination with imaging studies, physical examination, and medical history to monitor response to treatment in women with metastatic disease where no other reasonable measure of disease is feasible.[11] (**Minicase 2.**)

CA 27.29 Antigen

Normal range <38 units/mL

Cancer antigen 27.29 (CA 27.29) is also defined by an assay using a monoclonal antibody that detects circulating mucin antigen in blood.[9] It is a newer test than CA 15-3 but has the same clinical indications. CA 27.29 is used only in combination with other clinical factors such as imaging studies, physical examination, and medical history to monitor response to treatment in patients with metastatic breast cancer, but it is not useful as a screening test or for the detection of recurrence after primary therapy in early stage disease.[9]

CA 125 Antigen

Normal range: <35 units/mL

Cancer antigen 125 (CA 125 antigen) is a protein, which is usually found on cells that line the pelvic organs and peritoneum. It may also be detected in the blood of women with ovarian cancer and those with adenocarcinoma of the cervix or fallopian tubes. It may be elevated in nonmalignant conditions including endometriosis, ovarian cysts, liver disease, and pregnancy, and occasionally in many other types of cancer.[11] It is not, however, elevated by mucinous epithelial carcinomas of the ovaries. Levels of CA 125 also increase during menstruation and are lower at the luteal phase of the cycle. Levels are lower in women who use systemic contraceptives and also decline following menopause.[11]

CA 125 is assessed using a blood sample collected in a red top tube. The sample should be refrigerated within two hours of collection. The level of CA 125 in the serum has been reported to correlate with the likelihood of malignancy, with levels >65 units/mL strongly associated with the presence of a malignancy. However, such levels should not be considered diagnostic.[11,12] Several studies evaluating serial levels of CA 125 in healthy women have shown that serum levels may start to rise one to five years before the detection of ovarian cancer.[11] It does not, however, have sufficient sensitivity to be recommended as a routine screening test for ovarian cancer in asymptomatic women. The sensitivity in early stage ovarian cancer (before symptoms are usually evident) is believed to be <60%; thus, many cases would not be detected. Using CA 125 levels with other tests such as transvaginal ultrasound has been investigated to increase the utility of CA 125. However, using transvaginal ultrasound in patients with elevated CA 125 levels does not appear to increase the detection of early tumors and the routine use of the combination is not recommended.[11,13] However, recent data of the largest published prospective screening

MINICASE 2

A Case of Utilizing Tumor Markers for Breast Cancer

Sarah H., a 41-year-old white female, is recently diagnosed with breast cancer. She presents for her first scheduled routine mammogram, and a small lump is detected in her left breast. A fine-needle biopsy is done, and the lump is found to be positive for breast cancer. A complete workup determines that this is local disease, and she is diagnosed with stage II breast cancer. Additional medical history is unremarkable, and she only takes seasonal allergy medicine and drospirenone/ethinyl estradiol oral contraceptives.

A review of systems is noncontributory. Her physical examination is pertinent for a small lump palpable on the left breast near her nipple. Her cancer is evaluated for the presence of tumor markers, and the pathology shows ER/PR = positive and HER2 = 2+ on IHC.

QUESTION: How will these markers be evaluated and used to make treatment decisions in this patient? Are there any other tumor markers or tests you would recommend to be performed on her?

DISCUSSION: The two most important tumor markers in determining prognosis and treatment decisions are ER/PR status and HER2 status, and both were performed on this patient. Her ER/PR receptors were found to be positive. There are many ways to report ER/PR status with most being determined by IHC. Because the presence of even small amounts of ER/PR has been correlated with prognosis and the need for hormonal therapy, ER/PR status is commonly reported as either positive or negative. Because her ER/PR status is positive, she will benefit from hormonal therapy that targets the ER receptor and likely will be offered five years of tamoxifen based on her premenopausal status. The use of RT-PCR in determining ER/PR status could be done to confirm her ER/PR status.

She also has her HER2 status reported. Her value is 2+ as determined by IHC. This value is in the inconclusive range. Because HER2 status is critical in determining the benefit from anti-HER2 therapies (e.g., trastuzumab and lapatinib), inconclusive values require further workup. The confirmatory test that should be performed is a FISH assay. This test measures both the number of HER2 gene copies and provides a ratio of HER2/CEP 17 (also called *FISH ratio*). A positive test for HER2 gene amplification is a gene copy number >6 or a FISH ratio >2.2.

She should have this test performed. If positive, she will be offered trastuzumab as part of her adjuvant therapy. A positive result may also dictate part of her chemotherapy regimen; anthracyclines generally are recommended in HER2-positive patients. If she has a negative FISH test for HER2, then she will not receive adjuvant therapy and will instead receive a standard chemotherapy regimen followed by hormonal therapy.

Additional markers, such as CEA, CA 15-3, and CA 27.29, would not be useful in following this patient because she does not have metastatic disease, and these markers only are useful for determining progressive disease during treatment for metastatic breast cancer.

trial conducted to date demonstrated that using serial biomarker measurements doubled the number of screen-detected ovarian cancers.[14] This study used serial CA 125 levels in conjunction with a risk algorithm and compared this to serial CA 125 levels alone. The investigators concluded that using the algorithm in combination with serial CA 125 levels increased the number of cancers detected at screening.[14] The impact of this on ovarian cancer mortality is still unknown. Some advocate that rising serial CA 125 levels could be used as a trigger to do more extensive (and often costly) screening tests in high-risk women; however, this approach has not proven beneficial and may result in unacceptable morbidity in women at average risk for ovarian cancer.

Most often CA 125 is measured to monitor for evidence of disease recurrence or residual disease in women who have undergone surgical resection of ovarian cancer.[11] This indication is useful in women whose tumors expressed CA 125 prior to surgery. For women who have undergone a tumor debulking operation prior to chemotherapy, a level measured approximately three weeks after surgery correlates with the amount of residual tumor mass and is predictive of overall survival.[15] Serial levels during and following chemotherapy are used to monitor response to treatment, disease progression, and prognosis. However, many women, with CA 125 levels that have returned to the normal reference range during treatment still have residual disease as determined by a second-look laparotomy to pathologically evaluate the disease.[16] A more rapid decline of serum CA 125 during treatment has been associated with a more favorable prognosis.[11,17,18] Nadir values <10 units/mL predict improved survival, and increases in CA 125 from the nadir (even when below 35 units/mL) may be used to predict disease progression.[16,19] Failure of the CA 125 level to decline may also be used to identify tumors that are not responding to chemotherapy and an increase usually indicates progression.[19] However, a large European trial in over 1400 women failed to demonstrate an improvement in survival in treating women based on rising CA 125 levels alone.[20] Additional trials are ongoing to confirm these results. Subsequently, rising CA 125 levels, without any other evidence of disease, requires careful clinical interpretation to determine if patients require treatment interventions.

Human Chorionic Gonadotropin

Normal range: serum <5 mIU/mL (<5 IU/L)

Human chorionic gonadotropin (hCG) is a glycoprotein consisting of α and β subunits that is normally produced by the placenta during pregnancy.[21,22] Elevations in nonpregnant females and in males requires workup for malignant conditions. The β subunit is most commonly used as the determinant in both serum as a tumor marker and in urine tests for pregnancy. hCG is also commonly produced by tumors of germ cell origin including mixed germ cell or pure choriocarcinoma, tumors of the ovaries and testis, extragonadal tumors of germ cell origin, and gestational trophoblastic disease (e.g., hydatidiform mole). Occasionally islet cell tumors and gastric, colon, pancreas, liver, and breast carcinomas also produce hCG. Patients with trophoblastic disease often produce irregular forms of hCG that may or may not be recognized by the various automated assays and false-positive hCG immunoreactivity also has been reported. Newer highly specific and highly sensitive immunoassays have improved the reliability of this test. Radioimmunoassays and the DPC Immulite hCG test have been reported to have the greatest accuracy.

In patients with testicular cancer, elevated levels of hCG may be present with either seminomatous (1–25%) or nonseminomatous disease (10–70%), depending on the stage of disease, so the test is not sensitive enough to be used as a screening tool for asymptomatic patients.[21] hCG has an important prognostic role with levels >50,000 mIU/mL indicating a poor prognosis in nonseminomatous disease.[22] Most frequently, hCG is used to monitor response to therapy (i.e., an elevated level is evidence of residual disease following surgery) and to monitor for evidence of disease progression or recurrence during or after treatment.[22,23] hCG has a half-life of only 18–36 hours, so serum levels decline rapidly following therapeutic interventions, and failure to do so may indicate residual disease. [21-23]

CA 19-9 Antigen

Normal range: <37 units/mL

Cancer antigen 19-9 (CA 19-9) is an oncofetal antigen expressed by several cancers including pancreatic (71–93% of cases), gastric (21–42% of cases), and colon (20–40% of cases) carcinomas. Serum for this test is collected in a red top tube, and the sample is frozen for shipping for analysis. The sensitivity of the test is insufficient to be useful as a screening test for early stage diseases. It was originally developed for colon cancer monitoring, but is no longer recommended.[6] It is primarily used in pancreatic cancer to help discriminate benign pancreatic disease from cancer, to monitor for disease recurrence, and to assess the response to treatment interventions.[6,24] CA 19-9 levels have been used to evaluate the effectiveness of a chemotherapy regimen with rising values indicating a shorter patient survival and the possible need to change chemotherapy regimens.[25] An elevated CA 19-9 level is a poor prognostic factor in patients with inoperable pancreatic cancer.[26]

α-Fetoprotein

Normal range: <20 ng/mL

α-fetoprotein (AFP) is a glycoprotein made in the liver, gastrointestinal tract, and fetal yolk sac. It is found in high concentrations in the serum during fetal development (~3 mg/mL), and following birth it declines rapidly to <20 ng/mL. Serum for AFP evaluation should be collected in a red top tube and refrigerated until assayed using radioimmunoassay. It is elevated in about 70% of patients with hepatocellular carcinoma, 50–70% of patients with testicular nonseminomatous germ cell tumors, and occasionally in patients with other tumors such as ovarian germ cell, pancreatic, gastric, lung, and colon cancers.[22] Nonmalignant conditions that may be associated with increased levels of AFP include pregnancy, hepatitis, and cirrhosis. In patients with nonseminomatous germ cell tumors, the level of AFP serum concentrations seems to correlate with the stage of the disease.[22] In some parts of the world, AFP is used as a screening test for hepatocellular carcinoma in patients who are positive for HBsAg, and, therefore, are at increased risk for hepatocellular carcinoma. In the United States, however, AFP is used primarily to assist in the diagnosis of hepatocellular carcinoma. AFP levels >1000 ng/mL are common in patients with hepatocellular carcinoma.[27,28]

AFP levels also are used to monitor patients with hepatocellular carcinoma or ovarian and testicular germ cell tumors for disease progression or recurrence and to assess the impact of treatment interventions. The serum half-life of AFP is five to seven days, and usually an elevation of the serum level for more than seven days following surgery is an indication that residual disease was left behind.[24] Following successful treatment for nonseminomatous germ cell tumors of the testis, hCG, and AFP are repeated every one or two months during the first year, every two or three months during the second year, and less frequently thereafter along with physical exams and chest x-rays.[24] Increases in these serum tests are considered an indication for further treatment such as chemotherapy. Rising levels in patients receiving chemotherapy indicate that therapy should be changed, whereas declining levels predict a more favorable outcome.[22]

β2-Microglobulin

Normal range <2.5 mcg/mL

β2-microglobulin (B_2M) is a protein found on the surface of lymphocytes as well as in small quantities in the blood and urine. Elevations of B_2M may be seen in lymphoproliferative disorders including multiple (plasma cell) myeloma, chronic lymphocytic leukemia lymphoma. B_2M is renally excreted and may be elevated in nonmalignant conditions such as renal failure.[29]

B_2M is a reflection of tumor mass in multiple myeloma and is considered standard for the measurement of tumor burden. Measurement of serum B_2M is most commonly done in the workup of multiple myeloma and is an important part of the staging and prognosis for that disease. Additionally, B_2M will be used to follow multiple myeloma patients for treatment efficacy with increases in B_2M potentially indicating progressive disease.[29] Patients with serum B_2M levels ≥5.5 mcg/mL are diagnosed as International Staging System stage III patients and have a median survival of 29 months.[30]

Estrogen and Progesterone Receptor Assays

The levels of *estrogen receptor* (ER) and *progesterone receptor* (PR) in biopsy tissue from breast cancers predict both the natural history of the disease and the likelihood that the tumor will respond to hormonal manipulations. This test is not a blood test but requires tissue from the cancer obtained by a relatively noninvasive biopsy. ER status is also a prognostic factor with ER-negative tumors having a worse prognosis than ER-positive ones. For over 30 years, it has been the standard of practice to evaluate breast cancer tissue for these protein receptors and to use that information in directing therapeutic interventions. The relative concentration of hormone receptors can be determined using very small amounts of tumor tissue.

The current standard of practice is to measure each protein using immunohistochemistry (IHC); this method detects protein expression through an antibody-antigen interaction.[31] Although the method (e.g., antibody) used can vary, the biopsy is read by pathologists with the results reported as a percent positive cells. If ≥1% of cells are positive, one is considered to have ER-positive or PR-positive disease.[31] Biopsies scored 1–10% may be considered "weakly" positive, and risks and benefits of hormonal therapy should be discussed with patients, but the patient is still considered to have ER-positive or PR-positive disease. Because of the variety of methods to evaluate IHC staining and the intraobserver/interobserver variability, newer methods of measuring ER and PR status are under investigation including the use of reverse-transcriptase polymerase chain reaction (RT-PCR), which measures gene expression of ERs in tissue. Classification of ER-positive and PR- positive tumors are based on cutoff points of 6.5 and 5.5 units, respectively.[32] This test has demonstrated statistically significant superiority over IHC in predicting relapse in tamoxifen-treated, ER-positive patients in one retrospective trial.[32] Further validation of the test is needed before it becomes routinely used in clinical practice.[32]

Positive ER levels correlate with response to hormonal therapies including removal of the ovaries in premenopausal women or administration of an antiestrogen, such as tamoxifen, or an aromatase inhibitor such as anastrozole.[31] In addition, ER content in tumor biopsies correlates with benefit from adjuvant hormonal therapy following surgical removal of the tumor. After 15 years of follow-up in ER-positive breast cancer patients, tamoxifen decreased mortality by 9% in women who received five years of therapy.[33]

Human Epidermal Growth Factor Receptor 2

Human epidermal growth factor receptor 2 (HER2) is a transmembrane glycoprotein member of the epidermal growth factor receptor (EGFR) family with intracellular tyrosine kinase activity.[34] This group of receptors functions in the growth and control of many normal cells as well as malignant cells. The gene that encodes for HER2 is *c-erb B2*.[34] About 20% of samples from human breast cancers exhibit amplification of c-erb B2 or overexpression of HER2.[35]

There are many potential clinical applications based on HER2 status in breast cancer: (1) studies have described the role of HER2 in the prognosis of patients with breast cancer, with poor prognosis seen in overexpressers; (2) HER2 status may predict responsiveness to certain chemotherapy (e.g., anthracyclines, taxanes); (3) HER2 status may be used to predict resistance to other therapies (e.g., tamoxifen); and (4) HER2 status will predict benefit from anti-HER2 therapies, such as monoclonal antibodies (e.g., trastuzumab, ado-trastuzumab emtansine and pertuzumab) and tyrosine kinase inhibitors (e.g., lapatinib).[36-41]

However, the considerable variability in study design and the well-recognized heterogeneity of the disease itself have made interpretation difficult, and HER-status alone should not determine whether or not a woman should receive specific adjuvant therapy or whether endocrine therapy should be used.[10] The benefit of anthracycline therapy in the adjuvant setting is greatest in HER2 positive tumors and that determining the benefit of taxane-based therapy is inconclusive at this time.[10]

It is well-established that HER2 overexpression is predictive of a response to treatment with trastuzumab, ado-trastuzumab and pertuzumab, which are all monoclonal antibodies against HER2, and lapatinib a tyrosine kinase inhibitor of human epidermal growth factor receptor 1 (HER1) and HER2.[37,38,42,43] Therefore, it is necessary to evaluate all invasive breast cancers for HER2 status to select appropriate patients for these anti-HER2 therapies.[41]

Although a portion of the HER2 receptor can dissociate from the cell and be detected in the serum, biopsies of the tumor are routinely used to evaluate HER2 status. It can be measured for overexpression of the protein by IHC or by gene amplification, most commonly by using fluorescence in situ hybridization (FISH) assays.[41] Several commercial assays have been recommended to aid in the selection of patients for anti-HER2 therapy. Immunohistochemistry assays assess for the overexpression of the HER2 protein and a score of 0, 1+, 2+, or 3+ is reported. Clinical trials have demonstrated that those with a score of 0 or 1+ should be considered HER2 negative and do not benefit from anti-HER2 therapy, and those that are 3+ are HER2 positive and benefit from therapy.[37,41] A score of 2+ should be considered inconclusive and requires further evaluation with a FISH assay.

The FISH assay can be used as the initial test for HER2 positivity and is preferred by some groups due to decreased variability and increased ability to predict efficacy of therapies aimed at the HER2 receptor.[41] The FISH assay measures both the number of gene copies of HER2 gene as well as provides a ratio of HER2/CEP 17 (also called *FISH ratio*). Tumors are measured by the ratio of HER2 signals divided by the number of signals determined by the centromeric portion of chromosome 17 (CEP 17). A positive test for HER2 gene amplification is a gene copy number >4 or a FISH ratio >2. HER2 negative tumors are defined as a gene copy number <4 or FISH ratio <2, and FISH ratios between 1.8–2.2 are inconclusive. Additional cells should be scored and the results compared.[41] Only patients with FISH-positive tumors derive benefit from anti-HER2 therapy.[41]

In summary, the routine testing for HER2 with either IHC or FISH is recommended in all patients with invasive breast cancer, with FISH as the preferred method.[10,41] Patients who are HER2 positive benefit from trastuzumab (+/– pertuzumab) in the adjuvant setting and both trastuzumab (+/– pertuzumab) and lapatinib in the metastatic setting.[37,38,44] The use of HER2 testing to determine benefit of additional therapies (e.g., tamoxifen, anthracyclines, taxanes) is inconclusive at this time.[10,41] (Minicase 2.)

BCR-ABL

The identification of tumor markers in the pathogenesis of malignancy has led to the development of therapeutic strategies that specifically target the cause of the malignancy. By definition patients with chronic myelogenous leukemia (CML) possess the Philadelphia (Ph) chromosome that indicates the presence of the *BCR-ABL* fusion gene.[45,46] The *BCR-ABL* fusion gene also can be found in acute lymphoblastic leukemia and rarely in acute myeloid leukemia. *ABL* and *BCR* are normally found on chromosomes 9 and 22, respectively. The translocation of *ABL* and *BCR* t(9;22) in which both genes are truncated forming the characteristic *BCR-ABL* fusion gene on the Ph chromosome is diagnostic for CML and is present in all patients with the disease by definition.[45,46] The *BCR-ABL* gene encodes a protein with deregulated tyrosine kinase activity that has become the primary target for treating CML.

The Ph chromosome can be tested by the following three methods[45,46]: (1) conventional cytogenetic testing, in which bone marrow cells are aspirated and the individual chromosomes are examined for the presence of the Ph chromosome (the term *cytogenetic remission* has been developed to describe the elimination of the Ph chromosome on testing by this method after treatment); (2) FISH testing, which can be done on either blood or bone marrow cells (genetic probes are utilized to look for abnormal cells that contain the *BCR-ABL* gene); and (3) RT-PCR testing, which is the most sensitive test for monitoring response to therapy and counts the number of cells that contain the *BCR-ABL* gene (it can be done on either blood or bone marrow cells). Testing with RT-PCR is referred to as *molecular monitoring* and responses are called *molecular responses*. **Table 20-4** lists the response criteria for CML using cytogenetic and molecular monitoring.[45,46]

Therapies (e.g., imatinib, nilotinib, dasatinib, bosutinib, and ponatinib) have been developed that target the abnormal tyrosine kinase activity of the *BCR-ABL* gene.[47,4] As mentioned, efficacy is monitored by the elimination of the Ph chromosome (cytogenetic or molecular) and detection of increasing amounts of the *BCR-ABL* fusion gene often require adjustments in therapy.

TABLE 20-4. Criteria for Cytogenetic and Molecular Response in Patients with Chronic Myelogenous Leukemia

CYTOGENETIC RESPONSE	MOLECULAR RESPONSE
Complete: Ph +0% Partial: Ph +1–35%	Complete response indicates BCR-ABL transcript nonquantifiable and nondetectable
Minor: Ph +36–65% Minimal: Ph +66–95% None: Ph + >95%	Major molecular response defined as reduction of BCR-ABL:ABL ratio to 0.1% or less

Several mutations in the *BCR-ABL* gene have been identified that may predict response to the currently available tyrosine kinase inhibitors. Patients that present in advanced phase disease or have an inadequate or loss of response to tyrosine kinase inhibitors should undergo mutational analysis, so that the appropriate therapy may be selected. Patients with threonine-to-isoleucine mutation at codon 315 (T315I) were previously referred to stem cell transplantation due to a lack activity of the available tyrosine kinase inhibitors to this mutation. Ponatinib is now an FDA-approved tyrosine kinase inhibitor that is active against this mutation and patients that have the T315I mutation should be considered for this therapy. Failure with multiple therapies may lead to a referral for stem cell transplantation.[49]

Epidermal Growth Factor Receptor

Epidermal growth factor receptor (EGFR) (human epidermal growth factor receptor, HER1, c-erb B1) is a transmembrane glycoprotein member of the EGFR family with intracellular tyrosine kinase activity (TK) (same family as HER2). When EGFR receptors are activated, they support tumor growth by influencing cell motility, adhesion, invasion, survival, and angiogenesis. The gene that encodes for EGFR can have activating mutations in exon 18 through 21, but the ones of most interest influence the sensitivity or resistance to erlotinib, gefitinib and afatinib (EGFR tyrosine kinase inhibitors [TKi]). Class I mutations in exon 19 account for approximately 44% of all EGFR TK-activating mutations and a point mutation in exon 21 accounts for approximately 41% of EGFR TK-activating mutations. These mutations are most commonly found in adenocarcinoma of the lung from nonsmokers. They are also more common in Asians and females, which matches patient subset analysis from clinical trials with erlotinib. Approximately 15% of all U.S. patients with adenocarcinoma of the lung have one of these activating mutations. A secondary mutation in exon 20 (T790M) has been found to convey resistance to the current EGFR TKi treatments. Recent recommendations state that all patients who are being considered for first-line therapy with an EGFR TKi should have mutational analysis run on their tumor tissue.[50] EGFR mutational analysis can be performed with a number of different assays; however, standard testing for patient care includes PCR amplification and genetic sequencing of exon 18 through 21.[51]

V-Ki-Ras2 Kirsten Rat Sarcoma Viral Oncogene Homolog

V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRas) is an intracellular GTPase that plays an important role in signal transduction. Functionally, it works like an on/off switch that is downstream of a number of cell surface receptors including EGFR. When turned on, it conveys proliferative, growth, and survival signals; in the normal setting it turns off after conveying the activation signal. Mutated or oncogenic Ras performs the same function, but mutations in exon 1 (codons 12 and 13) lead to a permanently active Ras. Oncogenic Ras is found in

20–25% of all human tumors and in up to 90% of pancreatic cancers. This is obviously a target for drug development, but as of today no therapy has reached the market that inhibits this signal. It is, however, routinely used to select drug therapy, with patients having wild type (WT) (nonmutated) tumors more likely to respond to therapy. Mutated KRas is present in approximately 40% of colorectal tumors, where it conveys resistance to cetuximab and panitumumab. Current national guidelines and many payers require KRas mutational testing before giving either of these anti-EGFR monoclonal antibodies for colorectal cancer. Real-time PCR methods with fluorescent probes to common mutations in codon 12 and 13 are commonly used to determine if a KRas mutation exists; however, there are other methods including direct gene sequencing that can be used.[52]

V-Raf Murine Sarcoma Viral Oncogene Homolog B1

V-Raf murine sarcoma viral oncogene homolog B1 (BRAF) is a serine/threonine-specific protein kinase that plays an important role in signal transduction. It has activating mutations in 7–8% of all cancers and 40–60% of melanomas. The two most common mutations are V600E mutation (glutamic acid for valine substitution at amino acid 600) and the V600K mutation (valine to lysine substitution at amino acid 600).[53] This mutation means that the kinase is always turned on, signaling downstream partners in the mitogen-activated protein (MAP) kinase pathway, such as mitogen-activated extracellular kinase (MEK). Vemurafenib, a drug specifically designed to inhibit the mutated BRAF, is now available to treat melanoma in patients whose tumor contains this mutation. Concurrent with the approval of vemurafenib, the CobasC 4800 BRAF V600 mutation test was introduced, which utilizes real-time PCR to identify the V600E mutation in tumors; now other tests are available to detect V600E and V600K mutations. The prescribing information requires that the test be performed and the result be positive for the mutation before using the drug.[54] Dabrafenib, a BRAF inhibitor, and trametinib, a MEK 1 and 2 inhibitor, are available as combination treatment for patients with the V600E or V600K mutation; dabrafenib may be used as a single agent against patients with the BRAF V600E mutation.[55,56] BRAF mutations may also be found in non-Hodgkin lymphoma, colorectal cancer, thyroid carcinoma, non-small-cell lung carcinoma, and adenocarcinoma of lung.

Anaplastic Lymphoma Kinase

Anaplastic lymphoma kinase (ALK) is a fusion gene formed when the echinoderm microtubule-associated, protein-like 4 (EML4) is fused to *ALK*. The abnormal fusion protein promotes malignant cancer cell growth. This has recently become clinically relevant because a new drug, crizotinib, is highly effective for patients with lung cancer whose tumors contain this translocation. The mutation most commonly occurs in nonsmokers with lung adenocarcinoma, and it rarely occurs in combination with KRas or EGFR mutations. Although the mutation is found only in 2–7% of non-small-cell lung cancer patients, it should be routinely tested for due to significant improvement in outcomes with crizotinib and ceritinib that target this mutation. The rearrangement/fusion is usually detected with FISH; however, PCR and IHC can be used to identify the fusion gene or its protein product respectively.[57,58]

SUMMARY

To be clinically useful as a screening tool in asymptomatic individuals, tumor markers should be both sensitive and specific. Unfortunately, most of the tumor markers identified to date lack the sensitivity to be used in this capacity. In addition, many nonmalignant conditions cause elevations of these markers. Currently, only PSA is in widespread use as a screening tool when used along with the results of a digital rectal exam. Tumor markers are valuable to monitor for disease recurrence in patients who have undergone definitive surgery for cancers or to assess a patient's response to chemotherapy or other treatment interventions. In these situations, serial measurements of tests such as PSA for prostate cancer, CEA for colon cancer, hCG and AFP for testicular cancer, and CA 125 for ovarian cancer are considered standards in the follow-up care of patients with these malignancies. Increasingly tumor markers are being used to choose appropriate therapeutic strategies. Some tumor markers, such as HER2 and ER, are used as indicators of tumor sensitivity to therapies that target those receptors. Others such as the *BCR-ABL* gene found in CML patients, V600E and V600K mutations found in melanoma, and EGFR mutations found in lung cancer provide a specific target in which therapeutic strategies have been developed to inhibit the actual pathogenesis of the cancer.

LEARNING POINTS

1. ***If a patient with CML has a complete cytogenetic response, is the patient considered to be cured of leukemia and can the patient stop therapy?***

   ANSWER: Obtaining a complete cytogenetic response to therapies demonstrates that the patient is responding to treatment. However, molecular responses, in particular complete molecular responses, are the most sensitive test to determine if the Ph chromosome is still present. Unfortunately, reaching undetectable levels of BCR-ABL transcripts in a patient is not common and does not indicate cure; therefore, the patient should continue on therapy.

2. ***A patient with testicular cancer has his serum AFP level drawn 2 days after his surgery, and it is still elevated (250 ng/mL). Is this cause for concern?***

   ANSWER: Using serum tumor markers after surgery in testicular cancer is common, and the rate by which they decline has prognostic implications. However, because the serum half-life of AFP is 5–7 days, a level drawn so close to the surgery is of little value. In contrast, hCG has a half-life of only 18–36 hours. If hCG does not decrease within 2 days after surgery, this should be cause for concern.

3. *A patient is diagnosed with metastatic melanoma. Which pathway mutations should be checked prior to initiating therapy?*

ANSWER: The two most common mutations in metastatic melanoma are the V600E and the V600K mutations. This results in the constitutive activation of the mitogen-activated protein kinase pathway. Currently, we have therapies that inhibit the components BRAF and MEK in these mutation positive patients. Trials show that the combination of BRAF and MEK inhibitors are synergistic and may be used in combination if mutation positive.

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QUICKVIEW | Carcinoembryonic Antigen (CEA)

PARAMETER	DESCRIPTION	COMMENTS
Common reference range		
Adults	<2.5 ng/mL (<2.5 mcg/L)	
Pediatrics	Unknown	
Critical value	Yes, levels >10 ng/mL (>10 mcg/L) generally indicate cancerous process	
Inherent activity	Unknown	
Location		
Production	Intestine, pancreas, liver	Normally found during fetal development only; detected in serum of patients
Storage	Unknown	
Secretion/excretion	Unknown	
Causes of abnormal values		
High	Cancer (mainly colon), smoking, hepatitis, pancreatitis, peptic ulcer disease, hypothyroidism, ulcerative colitis, Crohn disease	Usually <10 ng/mL in nonmalignant conditions
Low	Not applicable	
Signs and symptoms		
High level	Not applicable	
Low level	Not applicable	
After event, time to....		
Initial elevation	Not applicable	
Peak values		
Normalization		
Causes of spurious results	Not applicable	
Additional info	Not reliable to screen for cancers because elevated in other conditions; can be used to monitor effectiveness of therapy in patients with cancer	

QUICKVIEW | CA 125

PARAMETER	DESCRIPTION	COMMENTS
Common reference range		
Adults	<35 units/mL	
Pediatrics	Unknown	
Critical value	Not applicable	
Inherent activity	Unknown	
Location		
Production	Protein found on cells of the pelvic organs and peritoneum	Detected in serum of patients
Storage	Unknown	
Secretion/excretion	Unknown	
Causes of abnormal values		
High	Cancer (mainly ovarian, cervical, and fallopian tube carcinomas), endometriosis, ovarian cysts, liver disease, pregnancy, menstruation	
Low	Luteal phase of cycle, patients on oral contraceptives, menopausal women	
Signs and symptoms		
High level	Not applicable	
Low level	Not applicable	
After event, time to....		
Initial elevation	Not applicable	
Peak values		
Normalization		
Causes of spurious results	Not applicable	
Additional info	Not reliable to screen for cancers because elevated in other conditions; can be used to monitor effectiveness of therapy in patients with ovarian cancer; rate of rise and fall of levels may indicate disease recurrence or residual disease	

QUICKVIEW | CA 15-3

PARAMETER	DESCRIPTION	COMMENTS
Common reference range		
Adults	<30 units/mL	
Pediatrics	Unknown	
Critical value	Not applicable	
Inherent activity	Unknown	
Location	Serum	
Production	Unknown	Antibody detects circulating mucin antigen secreted
Storage	Unknown	
Secretion/excretion	Secreted from breast tissue	
Causes of abnormal values		
High	Breast cancer, may be elevated in other cancers of lung, colon, ovary and pancreas origin and benign breast and liver disorders	
Low	Not applicable	
Signs and symptoms		
High level	Not applicable	
Low level	Not applicable	
After event, time to....		
Initial elevation	Not applicable	
Peak values		
Normalization		
Causes of spurious results	Not applicable	
Additional info	Mainly used in combination with other markers or in clinical trials	

QUICKVIEW | CA 27.29

PARAMETER	DESCRIPTION	COMMENTS
Common reference range		
Adults	<38 units/mL	
Pediatrics	Unknown	
Critical value	Not applicable	
Inherent activity	Unknown	
Location	Serum	
Production	Unknown	Antibody detects circulating mucin antigen secreted
Storage	Unknown	
Secretion/excretion	Secreted from breast tissue	
Causes of abnormal values		
High	Breast carcinoma, may be elevated in benign breast disorders	
Low	Not applicable	
Signs and symptoms		
High level	Not applicable	
Low level	Not applicable	
After event, time to….		
Initial elevation	Not applicable	
Peak values		
Normalization		
Causes of spurious results	Not applicable	
Additional info	Mainly used in combination with other markers or in clinical trials	

QUICKVIEW | Human Chorionic Gonadotropin (hCG)

PARAMETER	DESCRIPTION	COMMENTS
Common reference range		
Adults	<5 mIU/mL (<5 IU/L)	β subunit commonly measured, serum levels drawn when used as a tumor marker
Pediatrics	Unknown	
Critical value	Not applicable	
Inherent activity	Unknown	
Location		
Production	Made by cells of the placenta	Detected in patient serum and urine
Storage	Unknown	
Secretion/excretion	Secreted from the placenta or malignant germ cells	
Causes of abnormal values		
High	Pregnancy, mixed germ cell tumors, or choriocarcinoma of the testes or ovary, increased in other rare tumors	If elevated in males or in nonpregnant females, cancer is suspected
Low	Not applicable	
Signs and symptoms		
High level	Not applicable	
Low level	Not applicable	
After event, time to....		
Initial elevation	Not applicable	
Peak values		
Normalization		
Causes of spurious results	Not applicable	
Additional info	Most commonly used in testicular cancer as a prognostic factor as well as to monitor effects of treatment; levels >50 mIU/mL indicate a poor prognosis	

QUICKVIEW | CA 19-9

PARAMETER	DESCRIPTION	COMMENTS
Common reference range		
Adults	<37 units/mL	
Pediatrics	Unknown	
Critical value	Not applicable	
Inherent activity	Unknown	
Location		
Production	Pancreas, gastric cells, colon	Detected in patient serum
Storage	Unknown	
Secretion/excretion	Secreted from breast tissue	
Causes of abnormal values		
High	Pancreatic, gastric, and colon carcinomas; also in benign pancreatic disorders	
Low	Not applicable	
Signs and symptoms		
High level	Not applicable	
Low level	Not applicable	
After event, time to....		
Initial elevation	Not applicable	
Peak values		
Normalization		
Causes of spurious results	Not applicable	
Additional info	Only recommended to evaluate treatment response and recurrence in patients with pancreatic cancer	

QUICKVIEW | α-fetoprotein (AFP)

PARAMETER	DESCRIPTION	COMMENTS
Common reference range		
Adults	<20 ng/mL	
Pediatrics	Unknown	
Critical value	Not applicable	
Inherent activity	Unknown	
Location		
Production	Protein made normally during fetal and neonatal stages by liver, gastrointestinal tract, and yolk sac cells	Detected in patient serum; levels should decline after birth
Storage	Unknown	
Secretion/excretion	Unknown	
Causes of abnormal values		
High	Cancer (mainly liver and testicular); can be elevated in other cancers such as pancreatic, gastric, lung, and colon carcinomas; elevated in nonmalignant conditions including pregnancy, hepatitis, and cirrhosis	High results may be used to screen for liver cancer in parts of the world at increased risk for this malignancy
Low	Not applicable	
Signs and symptoms		
High level	Not applicable	
Low level	Not applicable	
After event, time to....		
Initial elevation	Not applicable	
Peak values		
Normalization		
Causes of spurious results	Not applicable	
Additional info	Only recommended to evaluate treatment response and recurrence in patients with testicular cancer	

QUICKVIEW | β2-Microglobulin (B_2M)

PARAMETER	DESCRIPTION	COMMENTS
Common reference range		
Adults	<2.5 mcg/mL	
Pediatrics	Unknown	
Critical value	Not applicable	
Inherent activity	Unknown	
Location	Protein found on surface of lymphocytes and other MHC I molecules	Also present in small amounts in urine and blood; level should decline after birth
Production	Unknown	
Storage	Unknown	
Secretion/excretion	Unknown	
Causes of abnormal values		
High	Multiple (plasma cell) myeloma, lymphoma, and in patients with renal failure	Renally excreted so elevated levels may indicate renal failure
Low	Not applicable	
Signs and symptoms		
High level	May see signs of renal failure	
Low level	Not applicable	
After event, time to....		
Initial elevation	Not applicable	
Peak values		
Normalization		
Causes of spurious results	Not applicable	
Additional info	Used in patients with multiple myeloma to assist in determining disease stage, prognosis, and response to treatment	

MHC = major histocompatibility complex.

QUICKVIEW | Estrogen and Progesterone Receptors

PARAMETER	DESCRIPTION	COMMENTS
Common reference range		
Adults	Not applicable	Not a normal serum laboratory value, only determined in breast biopsies; if >1% of cells are positive for the receptor, it is considered ER-positive or PR-positive
Pediatrics	Not applicable	
Critical value	Not applicable	
Inherent activity	Growth of breast and other hormone sensitive cells	
Location	Throughout the body (e.g., breast tissue, ovaries, bone)	Also present in small amounts in urine and blood; level should decline after birth
Production	Unknown	
Storage	Not applicable	
Secretion/excretion	Not applicable	
Causes of abnormal values		
High	Not applicable	It is unknown if the levels are higher in cancer, but they are checked to determine if blocking them with hormonal therapy will be useful
Low	Not applicable	
Signs and symptoms		
High level	May see signs of renal failure	
Low level	Not applicable	
After event, time to....		
Initial elevation	Not applicable	
Peak values		
Normalization		
Causes of spurious results	Not applicable	
Additional info	Antiestrogens (e.g., tamoxifen) and aromatase inhibitors (e.g., anastrozole) often given if these receptors are positive in women with breast cancer	

ER = estrogen receptor; PR = progesterone receptor.

QUICKVIEW | Human Epidermal Growth Factor Receptor 2 (HER2)

PARAMETER	DESCRIPTION	COMMENTS
Common reference range		
Adults	Considered positive by IHC if 3+ cells stain for HER2 or by FISH if *HER2* gene copy number >4 or FISH ratio >2	Not a normal serum laboratory value, only determined in breast biopsies; FISH preferred
Pediatrics	Not applicable	
Critical value	Not applicable	
Inherent activity	Protein involved in normal growth and development of cells by activating intracellular pathways that send growth signals to the nucleus	In cancer the growth signal is abnormal and amplified leading to uncontrolled proliferation of the cancerous cells
Location	Surface of many epidermal cells	Also present in small amounts in urine and blood; level should decline after birth
Production	Not applicable	
Storage	Not applicable	
Secretion/excretion	Not applicable	
Causes of abnormal values		
High	Cancer	Either number of receptors may be higher or there may be an increase in *HER2* gene copies indicating increased function of the gene
Low	Not applicable	
Signs and symptoms		
High level	Not applicable	
Low level	Not applicable	
After event, time to....		
Initial elevation	Not applicable	
Peak values		
Normalization		
Causes of spurious results	Not applicable	
Additional info	Anti-HER2 therapies (e.g., trastuzumab, ado-trastuzumab, pertuzumab, lapatinib) often given if positive	

FISH = fluorescence in situ hybridization; HER2 = human epidermal growth factor receptor 2; IHC = immunohistochemistry.

QUICKVIEW | BCR-ABL

PARAMETER	DESCRIPTION	COMMENTS
Common reference range		
Adults	Not applicable	This is an abnormal fusion gene that results from a genetic translocation producing a fusion mRNA normally not present in any significant amount unless a malignancy is present
Pediatrics	Not applicable	
Critical value	Not applicable	
Inherent activity	When present, causes abnormal growth of cells	Translocation results in an abnormal fusion protein with increased tyrosine kinase activity, which continually signals cells to grow
Location	Chromosome 22 resulting from t(9;22) translocation	Also present in small amounts in urine and blood; level should decline after birth
Production	Not applicable	
Storage	Not applicable	
Secretion/excretion	Not applicable	
Causes of abnormal values		
High	Cancer	
Low	Not applicable	
Signs and symptoms		
High level	Not applicable	
Low level	Not applicable	
After event, time to….		
Initial elevation	Not applicable	Rising levels of BCR-ABL mRNA correlate with increasing disease activity whereas falling levels are consistent with response to therapy
Peak values		
Normalization		
Causes of spurious results	Not applicable	
Additional info	Called the Philadelphia chromosome; levels of BCR-ABL mRNA should decrease with therapy, and failure to do so indicates treatment failure	

mRNA = messenger RNA.

QUICKVIEW | EGFR Mutation (exon 19 and 21)

PARAMETER	DESCRIPTION	COMMENTS
Common reference range		
Adults	Not applicable	This is a gene that codes for a transmembrane receptor; it does not normally contain any mutations
Pediatrics	Not applicable	
Critical value	Not applicable	
Inherent activity	When present, causes abnormal growth of cells	Mutation in lung cancer cells leads to perpetual signaling
Location	Located on chromosome 7p12—region of interest is exon 19 and 21h	
Production	Not applicable	
Storage	Not applicable	
Secretion/excretion	Not applicable	
Causes of abnormal values		
High	Not applicable	
Low	Not applicable	
Signs and symptoms		
High level	Not applicable	
Low level	Not applicable	
After event, time to….		
Initial elevation	Not applicable	
Peak values		
Normalization		
Causes of spurious results	Not applicable	
Additional info	The presence of a mutation in exon 19 or 21 in lung cancer indicates a higher likelihood of response to erlotinib, afatinib or gefitinib	

QUICKVIEW | KRas Mutation

PARAMETER	DESCRIPTION	COMMENTS
Common reference range		
Adults	Not applicable	This is a gene that codes for a GTPase that is a binary switch in cell signaling; it does not normally contain any mutations—when mutations are not present it is referred to as WT
Pediatrics	Not applicable	
Critical value	Not applicable	
Inherent activity	When present, causes abnormal growth of cells	Mutation in colorectal cancer cells leads to perpetual signaling and resistance to monoclonal antibodies targeting EGFR
Location	Chromosome 12p12— region of interest is exon 1 (codon 12 and 13)	
Production	Not applicable	
Storage	Not applicable	
Secretion/excretion	Not applicable	
Causes of abnormal values		
High	Mutation common in lung adenocarcinoma, mucinous adenoma, ductal carcinoma of the pancreas, and colorectal carcinoma	
Low	Not applicable	
Signs and symptoms		
High level	Not applicable	
Low level	Not applicable	
After event, time to....		
Initial elevation	Not applicable	
Peak values		
Normalization		
Causes of spurious results	Not applicable	
Additional info	Cetuximab and panitumumab only should be used for patients with colorectal cancer with WT KRas tumors	

EGFR = epidermal growth factor receptor; KRas = V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; WT = wild type.

QUICKVIEW | BRAF Mutation

PARAMETER	DESCRIPTION	COMMENTS
Common reference range		
Adults	Not applicable	This is a gene that codes for a kinase involved in cell signaling through the MAP kinase pathway; it does not normally contain any mutations
Pediatrics	Not applicable	
Critical value	Not applicable	
Inherent activity	When present, causes abnormal growth of cells	Mutation in cancer cells leads to perpetual signaling
Location	Chromosome 7q34—mutation of interest is at amino acid 600 (BRAF V600E or V600K)	
Production	Not applicable	
Storage	Not applicable	
Secretion/excretion	Not applicable	
Causes of abnormal values		
High	Mutation common in non-Hodgkin lymphoma, colorectal cancer, malignant melanoma, thyroid carcinoma, non-small-cell lung carcinoma, and adenocarcinoma of lung	
Low	Not applicable	
Signs and symptoms		
High level	Not applicable	
Low level	Not applicable	
After event, time to....		
Initial elevation	Not applicable	
Peak values		
Normalization		
Causes of spurious results	Not applicable	
Additional info	Patients with malignant melanoma should receive only vemurafenib and dabrafenib if they have a tumor with the V600E mutation or if the combination of dabrafenib and trametinib Is used, a V600E or V600k mutation should be present	

MAP = mitogen-activated protein.

QUICKVIEW | ALK Mutation

PARAMETER	DESCRIPTION	COMMENTS
Common reference range		
Adults	Not applicable	This is a fusion gene between EML4-and ALK; the resulting protein promotes cancer growth through increased kinase signaling activity; cells do not normally contain this gene fusion
Pediatrics	Not applicable	
Critical value	Not applicable	
Inherent activity	When present, causes abnormal growth of cells	Mutation in cancer cells leads to increased signaling
Location	Chromosome 2 contains the genes for EML4 and ALK—mutation of interest is translocation/fusion gene EML4-ALK	
Production	Not applicable	
Storage	Not applicable	
Secretion/excretion	Not applicable	
Causes of abnormal values		
High	Mutation most commonly found in adenocarcinoma of the lung carcinoma in nonsmokers	
Low	Not applicable	
Signs and symptoms		
High level	Not applicable	
Low level	Not applicable	
After event, time to….		
Initial elevation	Not applicable	
Peak values		
Normalization		
Causes of spurious results	Not applicable	
Additional info	Patients with metastatic non-small-cell lung cancer should receive only crizotinib or ceritinib if they have a tumor with the ALK rearrangement	

ALK = anaplastic lymphoma kinase.

PART III

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INTERPRETING PEDIATRIC LABORATORY DATA

Donna M. Kraus

OBJECTIVES

After completing this chapter, the reader should be able to

- Define the various pediatric age group terminology
- Discuss general pediatric considerations as they relate to blood sampling
- Describe how pediatric reference ranges are determined
- Discuss the age-related physiologic differences that account for variations by age in the normal reference ranges for serum sodium, potassium, bicarbonate, calcium, phosphorus, and magnesium
- List common pediatric causes of abnormalities in the electrolytes and minerals listed above
- Explain why age-related differences in serum creatinine and kidney function tests occur
- Discuss the age-related differences that occur in serum albumin, liver enzyme tests, and bilirubin
- Describe what is meant by the physiologic anemia of infancy and explain how it occurs

The interpretation of laboratory data in the pediatric patient population can be complex. Compared to adults, the pediatric population is much more dynamic. Alterations in body composition, organ function, and physiologic activity accompany the normal processes of maturation and growth that occur from birth through adolescence. These alterations can result in different normal reference ranges in pediatric patients for various laboratory tests. Pediatric patients not only have different normal laboratory values compared to adults, but also normal laboratory values may differ in various pediatric age groups. It is important for the clinician to understand the reasons for these different, commonly accepted reference ranges and to use age-appropriate reference ranges when providing pharmaceutical care to pediatric patients.

The measurement of substances in neonates, infants, and young children is further complicated by the patient's smaller physical size and difficulty in obtaining blood and urine samples. The smaller blood volume in these patients requires blood samples to be smaller; thus, special microanalytical techniques must be used. Additionally, in the neonate, substances that normally occur in higher amounts in the blood—such as bilirubin, lipids, and hemoglobin—may interfere with certain assays. This chapter will briefly review pertinent general pediatric principles and focus on the different age-related factors that must be considered when interpreting commonly used laboratory data in pediatric patients.

GENERAL PEDIATRIC CONSIDERATIONS

Knowledge of pediatric age group terminology is important to better understand age-related physiological differences and other factors that may influence the interpretation of pediatric laboratory data. These terms are defined in **Table 21-1** and will be used throughout this chapter.[1,2]

The interpretation of any patient's laboratory data must be viewed in light of the patient's clinical status. This includes the patient's symptoms, physical signs of disease, and physiologic parameters, such as respiratory rate, heart rate, and blood pressure. For example, an elevated $PaCO_2$ from an arterial blood gas may be clinically more significant in a patient who is extremely tachypneic (perhaps indicating impending respiratory failure) compared to a patient whose respiratory rate is mildly elevated. Thus, it is important to know the relative differences in physiologic norms that occur in the various pediatric age groups.

Normal respiratory rates are higher in neonates and young infants compared to children, adolescents, and adults. The average respiratory rate of a newborn is 60 breaths/min at one hour after birth, but 30–40 breaths/min at greater than six hours after birth. Mean respiratory rates of infants and young children <2 years of age (25–30 breaths/min) continue to be higher than in children 3–9 years of age (20–25 breaths/min) and adolescents (16–20 breaths/min).[1]

Normal heart rates follow a similar pattern with higher heart rates in neonates and young infants, which then slowly decrease with increasing age through adolescence. For example, the mean heart rate of a newborn is 120–160 beats/min and that of a one-month-old infant is 145 beats/min, while the mean heart rate for a one-year-old is 120 beats/min and that of a 12-year-old is 85 beats/min.[3]

TABLE 21-1. Definition of Age Group Terminology[a]

Gestational age (GA)	The time from conception until birth; more specifically, GA is defined as the number of weeks from the first day of the mother's LMP until the birth of the baby; GA at birth is assessed by the date of the LMP and by physical and neuromuscular examination (e.g., New Ballard Score)
Postnatal age (PNA)	Chronological age since birth
Postmenstrual age (PMA)	Postmenstrual age is calculated as gestational age plus postnatal age (PMA = GA + PNA)
Neonate	A full-term newborn 0–28 days PNA; some experts may also apply this terminology to a premature neonate who is >28 days PNA but whose PMA is ≤42–46 wk
Premature neonate	Neonate born at <37 wk GA
Full-term neonate	Neonate born at 37 wk 0 days to 41 wk 6 days (average ~40 wk) GA
Infant	1 mo (>28 days) to 12 mo of age
Child/children	1–12 yr of age
Adolescent	13–18 yr of age
Adult	>18 yr of age

LMP = last menstrual period.

[a] The term *postconceptional age* (PCA; age since conception) is no longer recommended for use in clinical pediatrics.[2] However, this term may be found in pediatric literature. Traditionally, PCA was defined as GA + PNA. Because the exact time of conception is not generally known (except in cases of assisted reproductive technology) and GA is calculated as above (according to the mother's LMP), PMA is considered a more accurate term to use. When PCA is used in the pediatric literature, it should be defined within the article where it is used.

Source: Reproduced, with permission, from reference 1.

In pediatric patients, normal blood pressure values vary according to age, gender, and percentile height of the patient.[4,5] Blood pressures are lower in neonates and increase throughout infancy and childhood. For example, typical blood pressures for a full-term newborn would be in the range of 65–95 systolic and 30–60 diastolic. The normal blood pressure (blood pressure <90th percentile) for a one-year-old girl of average height (50th percentile height) would be <100/54, while that of a 15-year-old girl of average height would be <123/79. Blood pressures are slightly different for girls compared to boys and are higher in taller children. Appropriate references should be consulted to obtain normal blood pressure values when providing clinical care to pediatric patients.[1,4,5]

In addition to age-related physiologic differences in respiratory rates, heart rates, and blood pressures, age-related changes in body composition (e.g., fluid compartments), cardiac output, organ perfusion, and organ function also exist. These age-related changes may result in different normal laboratory values for pediatric patients compared to adults. For example, age-related changes in fluid compartments affect normal laboratory values for serum electrolytes, as discussed in the Serum Electrolytes and Minerals section below. Being aware of the normal laboratory values for age is important for proper monitoring of efficacy and toxicity of pediatric drug therapy.

Pediatric Blood Sampling

The smaller physical size of pediatric patients makes it more difficult to obtain blood samples. In general, venipuncture techniques used in adults can be utilized in older children and adolescents. However, vacuum containers used for blood sampling may collapse the small veins of younger children and are not recommended in these patients.[6] Capillary puncture (also called *microcapillary puncture* or *skin puncture*) is used in patients with small or inaccessible veins. Thus, it is the blood sampling method of choice for premature neonates, neonates, and young infants. Because this method also helps preserve total blood volume, it may also be beneficial to use in infants and small children who require multiple blood tests.[7]

The physical sites that are used for capillary puncture include the heel, finger, great toe, and ear lobe.[6,7] The preferred site in neonates is the medial or lateral portion of the plantar surface of the heel. The medial surface of the great toe may also be used. The central area of the foot is avoided because of the risk of damage to the calcaneus bone, tendons, nerves, and cartilage. Heel sticks (capillary puncture of the heel) are often used in neonates and younger infants, while fingersticks may be used in children and adults. The earlobe is never used for capillary puncture for neonates and infants but may be used as a "site of last resort" in older children and adults.[7]

Because capillary and venous blood are similar in composition, the capillary puncture method may be used to obtain samples for most chemistry and hematology tests.[7] However, differences may occur between venous and capillary blood for certain substances such as glucose, calcium, potassium, and total protein. For example, glucose concentrations may be 10% higher when the sample is collected by capillary puncture compared to venipuncture.[6] In addition, improper capillary puncture sample collection may result in hemolysis or introduction of interstitial fluid into the specimen. This may result in higher concentrations for potassium, magnesium, lactate dehydrogenase, and other substances. Therefore, using the proper procedure to collect blood by the capillary puncture method is essential. It is also important that the site of capillary puncture be warmed prior to sample collection, especially for blood gas determinations.[6] Complications of capillary puncture include infection, hematoma, and bruising.

The size of the blood sample is an important issue to the pediatric clinician. Compared to adults, pediatric patients have

TABLE 21-2. Total Blood Volume by Age Group

AGE	EXAMPLE WEIGHT (kg), AGE	APPROXIMATE TOTAL BLOOD VOLUME (mL/kg)[a]	ESTIMATED TOTAL BLOOD VOLUME (mL)
Premature infant	1.5	89–105	134–158
Term newborn	3.4	78–86	265–292
1–12 mo	7.6 (6 mo)	73–78	555–593
1–3 yr	12.4 (2 yr)	74–82	918–1017
4–6 yr	18.2 (5 yr)	80–86	1456–1565
7–18 yr	45.5 (13 yr)	83–90	3777–4095
Adults	70.0	68–88	4760–6160

[a]Approximate total blood volume information compiled from reference 8.

Source: Reproduced, with permission, from Taketomo CK, Hodding JH, Kraus DM. Pediatric and neonatal dosage handbook. 22nd ed. Hudson, OH: Lexi-Comp Inc; 2015.

a much smaller total blood volume (**Table 21-2**). For example, a full-term newborn of average weight (3.4 kg) has an approximate total blood volume of 78–86 mL/kg, or about 265–292 mL total.[1,8] However, a 70-kg adult has an estimated total blood volume of 68–88 mL/kg or 4760–6160 mL total. If a standard 10-mL blood sample were to be drawn from a pediatric patient, it would represent a much higher percent of total blood volume compared to an adult. Therefore, the smaller total blood volume in pediatric patients requires blood sample sizes to be smaller. This issue is further complicated in newborns because their relatively high hematocrit (approximately 60% or higher) decreases the yield of serum or plasma from the amount of blood collected. Microanalytical techniques have reduced the required size of blood samples. However, critically ill, pediatric patients may require multiple or frequent blood sample determinations. Thus, it is essential to plan pediatric laboratory tests, especially in the neonate and premature neonate, to avoid excessive blood drawing.

Substances that normally occur in higher amounts in the blood of neonates, such as bilirubin, lipids, and hemoglobin, may interfere with certain assays. Hyperbilirubinemia may occur in premature and term neonates. High bilirubin concentrations may produce falsely low creatinine or cholesterol values when measured by certain analytical instruments.[6] Neonates, especially those that are born prematurely, may have lipemia when receiving intravenous (IV) fat emulsions. Lipemia may interfere with spectrophotometric determinations of any substance or with flame photometer determinations of potassium and sodium. Newborns have higher hemoglobin values and hemoglobin may interfere with certain assays. For example, hemolysis and the presence of hemoglobin may interfere with bilirubin measurements. Therefore, it is important to ensure that the assay methodology selected for measurement of substances in neonatal serum or plasma is not subject to interference from bilirubin, lipids, or hemoglobin.

Pediatric Reference Ranges

Various methods can be used to determine reference ranges, and each method has its own advantages and disadvantages. In adults, reference ranges are usually determined by obtaining samples from known healthy individuals. The frequency distribution of the obtained values are assessed and the extreme outliers (e.g., 0–2.5th percentile and 97.5–100th percentile) are excluded. This leaves the values of the 2.5–97.5th percentiles to define the reference range.[9] However, it also labels the 0–2.5th percentile and 97.5–100th percentile values from the healthy individuals as being outside of the reference range. If the frequency distribution of the obtained values fall in a bell-shaped or Gaussian distribution, then the mean (or average) value plus or minus two standard deviations (SDs) can then be used to define the reference range. The mean value plus or minus 2 SDs includes 95% of the sample. This method labels 5% of the healthy individuals as having values that fall outside of the reference range.

In the pediatric population, however, one cannot easily obtain blood samples from known healthy individuals. Large sample sizes of healthy pediatric individuals that include an appropriate age distribution from birth to 18 years of age would be required. Furthermore, it may be considered unethical to obtain blood samples from healthy pediatric patients when these individuals cannot legally give informed consent and there is no direct benefit to these individuals of obtaining the blood sample. Therefore, many pediatric reference ranges are determined by using results of tests from hospitalized sick pediatric patients and applying special statistical methods.[9] The statistical methods are designed to remove outliers and to distinguish the normal values from the values found in the sick patients. Obviously, problems in determining the true reference range may arise, especially when overlap between values from the diseased and nondiseased population occurs.

As in adults, many factors can influence the pediatric reference range including the specific assay methodology used, type of specimen analyzed, specific population studied, nutritional status of the individual, time of day the sample is obtained, timing of meals, medications taken, and specific patient demographics (age, sex, height, weight, and body surface area [BSA]). These factors, if not properly identified, may also influence the determination of reference ranges. In addition, because pediatric reference ranges are typically established in hospitalized patients, concomitant diseases may also influence the determination of the specific reference range being studied. All of these factors, plus the greater heterogeneity (variance) observed in the pediatric population makes the determination of pediatric reference ranges more complex.

Pediatric studies that define reference ranges may not always give detailed information about factors that may have influenced the determination of the specific pediatric reference range. Furthermore, due to the variation in influencing factors, most published pediatric reference ranges are not in exact agreement with each other.[1,3,9-17] Some studies report reference ranges by age for each year, others by various age groups, and others only by graphic display. Thus, it makes it very difficult to ascertain standard values for pediatric reference ranges and to apply published pediatric reference ranges to one's own patient population.

The reference ranges listed in this chapter reflect a compilation from various sources and are meant to be general guidelines. Clinicians should consult with their institution's laboratory to determine the specific age-appropriate pediatric reference ranges to be used in their patient population.

Pediatric Clinical Presentation

In general, the clinical symptoms of laboratory abnormalities in pediatric patients are similar to those symptoms observed in adults. However, certain manifestations of symptoms may be different in pediatric patients. For example, central nervous system irritability due to electrolyte imbalances (such as hypernatremia) may manifest as a high-pitched cry in infants. Hypocalcemia is more likely to manifest as seizures in neonates and young infants (compared to adults) due to the immaturity of the central nervous system. Neonates may also have nonspecific or vague symptoms for many disorders. For example, neonates with sepsis, meningitis, or hypocalcemia, may have poor feedings, lethargy, and vomiting. In addition, young pediatric patients are unable to communicate symptoms they may be experiencing. Thus, although symptoms of laboratory abnormalities in pediatric patients are important, oftentimes the correct diagnosis relies on the physical exam and appropriate laboratory tests.

SERUM ELECTROLYTES AND MINERALS

The homeostatic mechanisms that regulate fluid, electrolyte, and mineral balance in adults also apply to the pediatric patient. However, several important age-related differences exist. Compared to adults, neonates and young infants have alterations in body composition and fluid compartments; increased insensible water loss; immature (decreased) renal function; and variations in the neuroendocrine control of fluid, electrolyte, and mineral balance.[18] In addition, fluid, electrolyte, and mineral intake are not controlled by the individual (i.e., the neonate or young infant), but are controlled by the individual's caregiver. These age-related physiologic differences can result in alterations in the pediatric reference range for several electrolytes and minerals and can influence the interpretation of pediatric laboratory data.

A large percent of the human body is comprised of water. Total body water (TBW) can be divided into two major compartments: intracellular water (ICW) and extracellular water (ECW). The ECW compartment consists of the interstitial water and the intravascular water (or plasma volume) (**Figure 21-1**). Both TBW and ECW, when expressed as a percentage of body weight, are increased in the fetus and the newborn (especially the premature neonate) and decrease during childhood with increasing age (**Figure 21-2**).[19,20] The TBW of

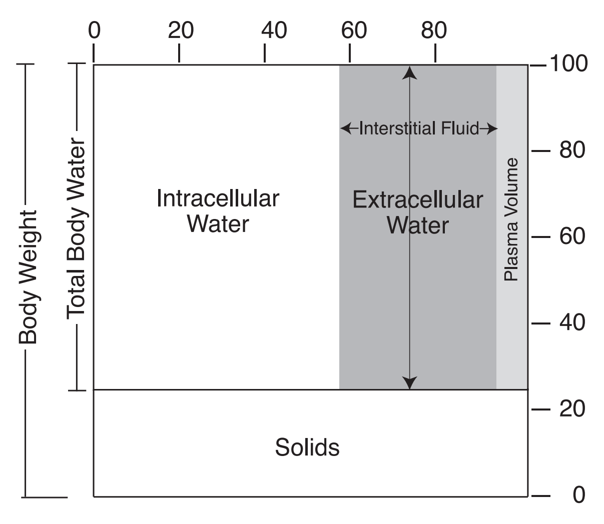

FIGURE 21-1. Distribution of body water in a term newborn infant. (Reproduced, with permission, from Bell EF, Oh W. Fluid and electrolyte management. In: MacDonald MG, Seshia MMK, Mullett MD, eds. Avery's neonatology: pathophysiology and management of the newborn. 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2005:363.)

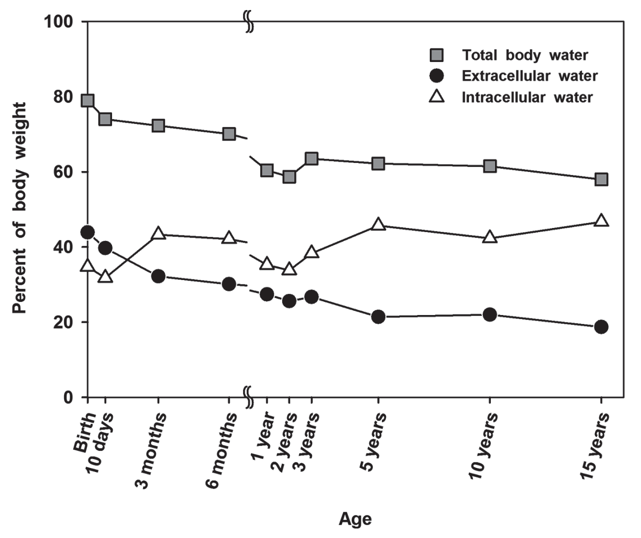

FIGURE 21-2. Changes in body water from birth to 15 years. (Data from reference 20.)

a fetus is 94% during the first month of gestation and decreases to 75% in a full-term newborn. The TBW of a preterm newborn may be 80%. The TBW decreases to approximately 60% by 6–12 months of age and to 55% in an adult. ECW is about 44% in a full-term newborn, 30% in a three- to six-month-old infant, 25% in a one-year-old, and 19% in an adult.[19,20] The decrease in TBW that is seen after birth is largely due to a contraction (or mobilization) of the ECW compartment. This mobilization is, most likely, the result of an increase in renal function that is seen after birth. The ICW compartment is lower at birth, increases slowly after birth, and is greater than ECW by about three months of age. It is important to note that the intake of water and electrolytes can influence these postnatal changes in TBW and the distribution between ECW and ICW.[18]

The electrolyte composition of ECW versus ICW is very different (**Figure 21-3**). Sodium is the major cation found in intravascular water (plasma volume) of the ECW. Potassium, calcium, and magnesium make up a much smaller amount of the intravascular cations. Chloride is the primary intravascular anion and bicarbonate, protein, and other anions comprise the balance. The electrolyte composition of the interstitial component of ECW is similar to the intravascular composition, but protein content is lower. Potassium and magnesium are the major cations found in ICW. Phosphate (organic and inorganic) is the primary intracellular anion, and bicarbonate makes up a smaller amount.[18]

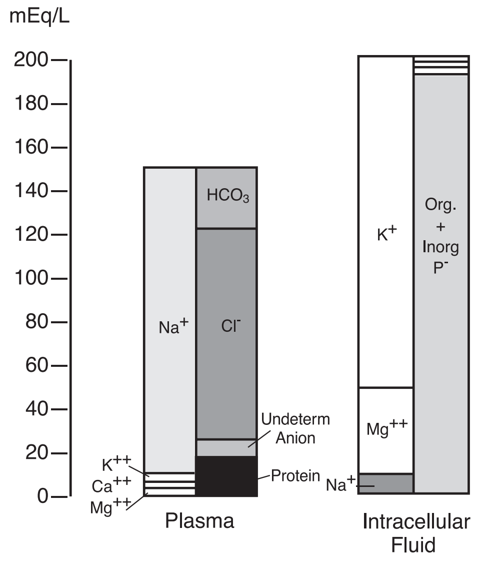

FIGURE 21-3. Ion distribution in the blood plasma, which represents extracellular fluid, and in the intracellular fluid compartment. (Reproduced, with permission, from Bell EF, Oh W. Fluid and electrolyte management. In: MacDonald MG, Seshia MMK, Mullett MD, eds. Avery's neonatology: pathophysiology and management of the newborn. 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2005:364.)

These compositional differences in ECW and ICW, along with the age-related differences in the amounts of these water compartments, can result in maturational differences in the amount of electrolytes per kg of body weight. For example, because premature neonates have a larger ECW compartment and ECW contains a higher amount of sodium and chloride, premature neonates contain a higher amount of sodium and chloride per kilogram of body weight compared to term neonates.[18] These principles are important to keep in mind when managing neonatal fluid and electrolyte therapy. One must also remember that the management of fluid and electrolyte therapy in the mother during labor can result in alterations in the newborn's fluid and electrolyte status. For example, if the mother is given too much fluid (i.e., too much free water) during labor, the newborn may be born with hyponatremia.[21]

Insensible water loss is the water that is lost via evaporation from the skin and through the respiratory tract.[18] Knowledge of the factors that influence insensible water loss in pediatric patients is important to estimate appropriate water intake and to assess electrolyte imbalances that may occur. Compared to adults, neonates and young infants have an increase in the amount of insensible water loss. This is primarily due to their increased surface area to body weight ratio and higher respiratory rate. Smaller newborns and those born at a younger gestational age (GA) have an even higher insensible water loss. This is related to their immature (thinner) skin, greater skin blood flow, and larger TBW. Many other factors increase insensible water loss, such as the environmental and body temperature, radiant warmers, phototherapy, motor activity, crying, and skin breakdown or injury. Congenital skin defects, such as gastroschisis, omphalocele, or neural tube defects will also increase insensible water loss. The use of high inspired or ambient humidity, plastic heat shields or blankets, occlusive dressings, and topical waterproof agents will decrease insensible water loss.

The primary functions of the kidney (glomerular filtration, tubular secretion, and tubular reabsorption) are all decreased in the newborn, especially in the premature newborn, compared to adults. These functions increase with GA at birth and with postnatal age (PNA). The decreased glomerular and tubular functions in the neonatal kidney result in differences in how the neonate handles various electrolyte loads and differences in the normal reference ranges for several electrolytes, as described below.

Sodium

Normal range[22]*:*
premature neonates (at 48 hr of life): 128–148 mEq/L (128–148 mmol/L)
neonates: 133–146 mEq/L (133–146 mmol/L)
infants: 139–146 mEq/L (139–146 mmol/L)
children: 138–145 mEq/L (138–145 mmol/L)
adults: 136–142 mEq/L (136–142 mmol/L)

Sodium is primarily excreted via the kidneys, but it also is excreted via stool and sweat.[23] Usually, unless diarrhea occurs, sodium loss in the stool is minimal. In children with cystic fibrosis, aldosterone deficiency, or pseudohypoaldosteronism, the sodium concentration in sweat is increased and higher sweat losses may contribute to or cause sodium depletion.

In neonates and young infants, the renal handling of sodium is altered compared to adults.[24,25] Differences in tubular reabsorption, aldosterone concentrations, and patterns of renal blood flow help to maintain a positive sodium balance, which is required for growth. In the neonate, sodium reabsorption is decreased in the proximal tubule but increased in the distal tubule. Aldosterone increases sodium reabsorption in the distal tubules, and plasma concentrations of renin, angiotensin II, and aldosterone are all increased in neonates. This increase in aldosterone may be a compensatory mechanism to help increase sodium reabsorption in the distal tubule. The pattern of renal blood flow is also different in the neonate. In adults, a larger amount of renal blood flow goes to the cortical area of the kidneys. However, in the neonate, the majority of renal blood flow goes to the medullary area, which is more involved with sodium conservation than excretion. These factors help the neonatal kidney to retain sodium, but also result in the neonate having a decreased ability to excrete a sodium load. Therefore, if an excessive amount of sodium is administered to a neonate, it will result in sodium retention with subsequent water retention and edema.

Although most infants are in a positive sodium balance, very low birth weight infants (birth weight <1.5 kg) are usually in a negative sodium balance.[24] This is due to their very immature kidneys and the larger amounts of sodium that are lost in the urine. These infants are at a higher risk of sodium imbalance and may require higher amounts of sodium, especially during the first weeks of life. Compared to adults, pediatric patients may be more susceptible to imbalances of sodium and water. This may be due to their higher amount of TBW and the common pediatric occurrence of causative factors such as diarrhea and dehydration.

Hyponatremia

In infants and children, *hyponatremia* is defined as a serum sodium <135 mEq/L, although slightly lower values would be considered acceptable for premature neonates and newborns.[23] As in adults, hyponatremia occurs in pediatric patients when the ratio of water to sodium is increased. This may occur with low, normal, or high amounts of sodium in the body; likewise, the amount of water in the body may be low (hypovolemic), normal (euvolemic), or high (hypervolemic). The causes of hyponatremia in pediatric patients are the same as in adults. However, certain causes may be more commonly seen in children.

In hypovolemic hyponatremia, both sodium and water have been lost from the body, but a higher proportion of sodium has been lost. The most common cause of hypovolemic hyponatremia in children is diarrhea due to gastroenteritis.[23] Emesis also can cause hyponatremia if hypotonic fluids are administered, but most children with emesis have either a normal serum sodium or hypernatremia. In addition to gastrointestinal (GI) losses, hypovolemic hyponatremia may also occur from losses of sodium through the skin (e.g., excessive sweating or burns), third space losses, and renal losses.

Renal sodium loss can occur in the pediatric population from a number of causes including thiazide or loop diuretics, osmotic diuresis, cerebral salt wasting, and hereditary or acquired kidney diseases. Cerebral salt wasting is thought to be due to hypersecretion of atrial natriuretic peptide, which causes renal salt wasting. This condition is usually seen in patients with central nervous system disorders such as head trauma, brain tumors, hydrocephalus, cerebral vascular accidents, neurosurgery and brain death.[26] Hereditary kidney diseases that can cause hypovolemic hyponatremia include juvenile nephronophthisis, autosomal recessive polycystic kidney disease, proximal (type II) renal tubular acidosis, 21-hydroxylase deficiency, and pseudohypoaldosteronism type I. Patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency have an absence of aldosterone. Aldosterone is needed for sodium retention and potassium and acid excretion in the kidneys. The lack of aldosterone in these patients produces hyponatremia, hyperkalemia, and metabolic acidosis. Patients with pseudohypoaldosteronism have elevated aldosterone serum concentrations, but the kidneys do not respond properly to aldosterone. A lack of response to aldosterone by the renal tubules may also occur in children with a urinary tract obstruction and acute urinary tract infection and result in hyponatremia.[23]

In euvolemic hyponatremia, patients have no real evidence of volume depletion or volume overload.[23] Usually, these patients have a slight decrease in total body sodium with an excess of TBW. Although some patients may have an increase in body weight (indicating volume overload), patients often appear clinically normal or have subtle signs of fluid overload. Causes of euvolemic hyponatremia include the syndrome of inappropriate antidiuretic hormone (SIADH), glucocorticoid deficiency, hypothyroidism, and water intoxication. Although SIADH is not common in children, it may occur in patients with central nervous system disorders or lung disease and tumors. Certain medications can cause an increase in antidiuretic hormone (ADH) secretion and are reviewed in Chapter 12.

Dilutional hyponatremia may commonly occur in hospitalized children who receive relatively large amounts of free water (e.g., hypotonic IV solutions). This may even occur when medications are diluted in 5% dextrose in water, for example, and administered as 50- or 100-mL IV rider bags or piggyback riders. Neonates and young infants are more prone to this water overload (due to their lower glomerular filtration rate [GFR] and limited ability to excrete water), and, thus, should receive medications diluted in smaller volumes of IV fluid. Other causes of hyponatremia due to water intoxication in pediatric patients include administration of diluted infant formula, tap water enemas, infant swimming lessons, forced water intake (child abuse), and psychogenic polydipsia.[23] (**Minicase 1.)**

In hypervolemic hyponatremia, both sodium and water are increased in the body, but there is a greater increase in water than sodium. Hypervolemic hyponatremia is typically observed in patients with congestive heart failure, cirrhosis, nephrotic syndrome, and chronic renal failure. It may

MINICASE 1

A Case of Hyponatremia and Seizures

Holly H., a four-day-old female, is currently in the neonatal ICU being treated with antibiotics for suspected sepsis. This morning she began having rhythmic clonic twitching of her lower extremities, fluttering of her eyelids, and repetitive chewing movements, which are consistent with seizure activity. She was born at 38 weeks of gestation to a mother with prolonged rupture of membranes (>72 hours). On the day of her birth, she was admitted to the neonatal ICU with an elevated temperature, tachycardia (HR 168), and a low WBC count (3.1×10^3 cells/mm^3). Blood and urine cultures were obtained, and antibiotics were started to treat her possible sepsis. Culture results are still pending. Medications include ampicillin 160 mg IV in 25 mL D5W as IV rider q 8 hr (150 mg/kg/day) and gentamicin 16 mg IV in 50 mL D5W as IV rider q 24 hr (5 mg/kg/day).

Her vital signs include BP 75/48 mm Hg, HR 131 beats/min, RR 32 breaths/min, and temperature 98.8 °F. Length is 49 cm (50th percentile for age), and weight is 3.2 kg (50th percentile for age). Laboratory data include sodium 120 mEq/L, potassium 4.1 mEq/L, chloride 99 mEq/L, total CO_2 20 mEq/L, BUN 8 mg/dL, SCr 0.5 mg/dL, and glucose 85 mg/dL.

QUESTION: What is the most likely cause of this patient's seizure activity and electrolyte imbalance? What other laboratory tests should be obtained to further assess her seizure disorder?

DISCUSSION: Electrolyte imbalance is a common cause of neonatal seizures. As in adults, hyponatremia may cause seizure activity in neonates and occurs when the ratio of water to sodium is increased. The total body content of sodium in patients with hyponatremia may be low, normal, or high, and the volume status may be hypovolemic, euvolemic, or hypervolemic. There are many causes of hyponatremia, but the most likely cause in this patient is the extra D5W that she received with her antibiotics. Dilutional hyponatremia may occur in neonates and young infants when medications are administered in excess fluids, such as IV riders of 5% dextrose in water. These patients are more prone to water overload due to their lower GFR and their limited ability to excrete water. Medications for these patients should be diluted in smaller amounts of IV fluid, so that excess fluid is not administered. To better define her sodium and volume status, her total fluid intake and output, type of IV fluids administered, changes in body weight, and other laboratory data need to be assessed. In addition, other causes of hyponatremia, such as meningitis and SIADH, should be ruled out. A low WBC count, as observed in Holly H., often occurs in neonates with a serious bacterial infection (see White Blood Cell Count section). Although the most likely cause of her seizure activity is her low serum sodium, her serum calcium, phosphorous, and magnesium levels also should be assessed because other electrolyte abnormalities can cause seizure activity.

also occur in patients with hypoalbuminemia or in patients with capillary leak syndrome due to sepsis.[23] These conditions decrease the patient's effective blood volume, either due to poor cardiac function or third spacing of fluid. The compensatory mechanisms in the body sense this decrease in blood volume; ADH and aldosterone are secreted and cause retention of water and sodium in the kidneys. A decrease in serum sodium occurs because the intake of water in these patients is greater than their sodium intake and ADH decreases water excretion.

Hyponatremia may also be caused by hyperosmolality (due to hyperglycemia or iatrogenic substances such as mannitol, sucrose, or glycine). For example, in pediatric patients with hyperglycemia during diabetic ketoacidosis, the high serum glucose concentration results in a high serum osmolality, which causes a shift of water from the intracellular space into the extracellular (intravascular) space. This shift of water has a dilutional effect on the serum sodium concentration, resulting in hyponatremia.[23] The decrease in serum sodium is proportional to the increased serum glucose concentration; for every 100 mg/dL increase in serum glucose above normal, the measured serum sodium declines by 1.6 mEq/L. The following equation can be used in pediatric patients with hyponatremia due to hyperglycemia to correct the measured (low) serum sodium concentration.

$$\text{Corrected serum sodium} = \text{Measured serum sodium} + [1.6 \times (\text{serum glucose} - 100\text{ mg/dL})/100]$$

The calculated corrected serum sodium concentration better reflects the patient's true ratio of total body sodium to TBW; however, it should be remembered that the calculated value is only an estimate.

Hypernatremia

In general for pediatric patients, *hypernatremia* is defined as a serum sodium concentration >145 mEq/L. As in adults, hypernatremia occurs in pediatric patients when the ratio of sodium to water is increased. This may occur with low, normal, or high amounts of sodium in the body. Hypernatremia may occur with excessive sodium intake, excess water loss, or a combination of water and sodium loss when the water loss exceeds the sodium loss.[23]

Excessive sodium intake or sodium intoxication may occur due to improperly mixed infant formulas, excess sodium bicarbonate administration, IV hypertonic saline solutions, intentional salt poisoning (e.g., child abuse), and ingestion of sodium chloride or seawater.[23] Neonates, especially premature newborns, and young infants can develop hypernatremia from excessive sodium due to the decreased ability of immature kidneys to excrete a sodium load. This becomes a problem especially in the premature neonate when IV sodium bicarbonate is used to correct a metabolic acidosis. Excess water loss resulting in hypernatremia may occur in pediatric patients due to diabetes insipidus, increased insensible water losses, or inadequate intake. Diabetes insipidus can be of central or nephrogenic origin and either type can be acquired or congenital. Also, certain drugs may cause diabetes insipidus (see Chapter 12).

Neonates may be predisposed to hypernatremia from increased insensible water losses, especially during the first few days of life. A normal physiologic contraction of the ECW occurs after birth, resulting in a net loss of water and sodium. In term infants, this may result in a weight loss of 5–10% during the first week of life. In premature newborns, the weight loss may be 10–20%. This water loss, plus the relatively large and variable insensible water loss in neonates, can complicate the assessment of fluid and sodium balance. More premature newborns may be at higher risk for hypernatremia, as they have a more pronounced contraction of ECW and higher insensible water loss.[27] The use of radiant warmers and phototherapy (used to treat hyperbilirubinemia) will further increase insensible water loss.

Inadequate water intake also can cause hypernatremia in pediatric patients. This may be due to the caregiver not administering enough fluids (e.g., child neglect or abuse, or ineffective breastfeeding). Ineffective breastfeeding may result in severe hypernatremic dehydration. Rarely, inadequate intake may be due to adipsia (absence of thirst).[23]

Hypernatremia, due to water losses greater than sodium losses, occurs in patients with water and sodium losses through the GI tract (e.g., diarrhea, emesis, nasogastric suctioning, and osmotic cathartics), skin (e.g., burns and excessive sweating), and kidneys (e.g., diabetes mellitus, chronic kidney disease, osmotic diuretics, and acute tubular necrosis [polyuric phase]). Hypernatremia is most likely to occur in infants or children with diarrhea who also have inadequate fluid intake due to anorexia, emesis, or lack of access to water.

It should be noted that due to the immaturity of the blood vessels in their central nervous system, premature neonates are especially vulnerable to the adverse effects of hypernatremia (e.g., intracranial hemorrhage). These patients are also at greater risk of adverse central nervous system effects if an elevated serum sodium is corrected too rapidly. Thus, maintaining a proper sodium balance in these patients is extremely important.

Potassium

Normal range[11,22]:
premature neonates (at 48 hr of life): 3–6 mEq/L (3–6 mmol/L)
neonates: 3.7–5.9 mEq/L (3.7–5.9 mmol/L)
infants: 4.1–5.3 mEq/L (4.1–5.3 mmol/L)
children: 3.4–4.7 mEq/L (3.4–4.7 mmol/L)
adults: 3.8–5 mEq/L (3.8–5 mmol/L)

Potassium is the major intracellular cation, and <1% of total body potassium is found in the plasma.[23] However, small changes in serum potassium can have large effects on cardiac, neuromuscular, and neural function. Thus, appropriate homeostasis of extracellular potassium is extremely important. Insulin, aldosterone, acid–base balance, catecholamines, and renal function all play important roles in the regulation of serum potassium. Serum potassium can be lowered quickly when potassium shifts intracellularly or more slowly via elimination by the kidneys.

The kidney is the primary organ that regulates potassium balance and elimination. Potassium undergoes glomerular filtration and almost all filtered potassium is then reabsorbed in the proximal tubule. Urinary excretion of potassium, therefore, is dependent on distal potassium secretion by the collecting tubules. Neonates and young infants, however, have a decreased ability to secrete potassium via the collecting tubules. Thus, the immature kidneys tend to retain potassium. This results in a positive potassium balance, which is required for growth (potassium is incorporated intracellularly into new tissues).[24,25] Potassium retention by the immature kidneys also results in higher serum potassium concentrations compared to the adult.[25]

Hypokalemia

Hypokalemia is defined as a serum potassium concentration <3.5 mEq/L. As in adults, a low serum potassium may occur in pediatric patients due to an intracellular shift of potassium, decreased intake, or increased output (from renal or extrarenal losses). An intracellular shift of potassium may be seen with alkalosis, β-adrenergic stimulation, or insulin treatment. Endogenous β-adrenergic agonists (such as epinephrine released during stress) and exogenously administered β-agonists (such as albuterol) stimulate the cellular uptake of potassium. Other causes of an intracellular shift of potassium seen in pediatric patients include overdoses of theophylline, barium intoxication, and glue sniffing (toluene intoxication). A falsely low potassium concentration can be reported in a patient with a very elevated white blood cell (WBC) count (e.g., a patient with leukemia) if the plasma sample is inappropriately stored at room temperature. This allows the WBCs to uptake potassium from the plasma resulting in a falsely low measurement.[23]

Most cases of hypokalemia in children are related to extrarenal losses of potassium due to gastroenteritis and diarrhea.[23] Hypokalemia due to diarrhea is usually associated with a metabolic acidosis, because bicarbonate is also lost in the stool. Adolescent patients with eating disorders may be hypokalemic due to inadequate intake of potassium, for example, in patients with anorexia nervosa. Adolescents with bulimia or laxative abuse may also have significant extrarenal losses of potassium.

Many causes of hypokalemia due to renal potassium loss exist. Medications commonly used in the pediatric population that are associated with hypokalemia due to renal potassium loss include loop and thiazide diuretics, corticosteroids, amphotericin B, and cisplatin (Chapter 12). Cushing syndrome, hyperaldosteronism, and licorice ingestion may also cause hypokalemia via this mechanism.

In the pediatric population, other causes of increased renal potassium loss, such as hereditary diseases, must be considered. Remember that many hereditary diseases are first diagnosed during infancy and childhood. Renal tubular acidosis (both distal and proximal types) may present with hypokalemia and metabolic acidosis. Patients with cystic fibrosis have greater losses of chloride in sweat. This may lead to metabolic alkalosis, low urine chloride, and hypokalemia. Certain forms of congenital adrenal hyperplasia may also lead to increased renal potassium excretion and hypokalemia. Other inherited renal diseases that are due to defects in renal tubular transporters, such as Bartter syndrome, may result in metabolic alkalosis, hypokalemia, and high urine chloride. Thus, unlike the adult population, hereditary diseases need to be considered when certain electrolyte abnormalities are not explained by common causes.

Hyperkalemia

In infants, children, and adults, *hyperkalemia* is defined as a serum potassium >5 mEq/L. Because a normal serum potassium is slightly higher in neonates and preterm infants, hyperkalemia is defined as a serum potassium >6 mEq/L in these patients. Hyperkalemia is one of the most alarming electrolyte imbalances because it has the potential to cause lethal cardiac arrhythmias.

As in adults, hyperkalemia in pediatric patients may be due to increased intake, an extracellular shift of potassium, or decreased renal excretion. Factitious hyperkalemia is very common in pediatric patients, due to the difficulty in obtaining blood samples. Hemolysis often occurs during blood sampling and potassium is released from red blood cells (RBCs) in sufficient amounts to cause falsely elevated test results. This may especially happen with improperly performed heel sticks (see section on Pediatric Blood Sampling). Potassium may also be released locally from muscles after prolonged tourniquet application or from fist clenching, which may also result in false elevations of measured potassium. A falsely elevated serum potassium also can be observed in patients with leukemia or extremely elevated WBC counts (usually >200,000/mm^3) due to the release of potassium from WBCs. Prompt analysis with measurement of a plasma sample usually avoids this problem.[23]

Hyperkalemia may occur due to extracellular shifts of potassium. During a metabolic acidosis, hydrogen ions move into the cells (down a concentration gradient), and in exchange, potassium ions move out of the cells into the extracellular (intravascular) space. This shift leads to a significant increase in serum potassium.

In older patients with fully developed (normal) renal function, hyperkalemia rarely results from increased intake alone. However, this may occur in patients receiving large amounts of oral or IV potassium or in patients receiving rapid or frequent blood transfusions (due to the potassium content of blood).[23] In patients with immature renal function or in those with renal failure, increased intake of potassium can also lead to hyperkalemia due to decreased potassium excretion.

Decreased renal excretion of potassium is the most common cause of hyperkalemia. Decreased potassium excretion occurs in patients with immature renal function, renal failure, primary adrenal disease, hyporeninemic hypoaldosteronism, renal tubular disease, and with certain medications.[23] Hyperkalemia is the most common life-threatening electrolyte imbalance seen in neonates. Because of the decreased ability of immature kidneys to excrete potassium, neonates, particularly premature neonates, may be predisposed to hyperkalemia. These patients also cannot tolerate receiving extra potassium. Hyperkalemia can be seen in premature infants, during the first three days of life, even when exogenous potassium is not given and when renal dysfunction is absent.[27] A rapid elevation in serum potassium is seen within the first day of life in more immature newborns. This hyperkalemia, which can be life-threatening, may be due to a shift of potassium from the intracellular space to the extracellular (intravascular) space, immaturity of the distal renal tubules, and a relative hypoaldosteronism.[28]

Acute or chronic renal failure in pediatric patients will decrease potassium excretion and may result in hyperkalemia. Several inherited disorders may also cause decreased potassium excretion and hyperkalemia in pediatric patients including certain types of congenital adrenal hyperplasia (e.g., 21-hydroxylase deficiency), aldosterone synthase deficiency, sickle cell disease, and pseudohypoaldosteronism (types I and II).[23] Medications used in pediatric patients that may also cause hyperkalemia include angiotensin-converting enzyme inhibitors, β_2-adrenergic antagonists, potassium-sparing diuretics, nonsteroidal anti-inflammatory agents, heparin, trimethoprim, and cyclosporine.

Serum Bicarbonate (Total Carbon Dioxide)

Normal range[22,25]*:*
preterm infants: 16–20 mEq/L (16–20 mmol/L)
full-term infants: 19–21 mEq/L (19–21 mmol/L)
infants–children 2 yr of age: 18–28 mEq/L (18–28 mmol/L)
children >2 yr and adults: 21–28 mEq/L (21–28 mmol/L)

The *total carbon dioxide concentration* actually represents *serum bicarbonate*, the basic form of the carbonic acid–bicarbonate buffer system (i.e., a low serum bicarbonate may indicate an acidosis). In addition to the buffer systems, the kidneys also play an important role in acid–base balance. The proximal tubule reabsorbs 85–90% of filtered bicarbonate. The distal tubule is responsible for the net secretion of hydrogen ions and urinary acidification.[29] Compared to adults, neonates have a decreased capacity to reabsorb bicarbonate in the proximal tubule, and, therefore, a decreased renal threshold for bicarbonate (the renal threshold is the serum concentration at which bicarbonate appears in the urine). The mean renal threshold for bicarbonate in adults is 24–26 mEq/L but only 18 mEq/L in the premature infant and 21 mEq/L in the term neonate. The renal threshold for bicarbonate increases during the first year of life and reaches adult values by about one year of age. Neonates also have decreased function of the distal tubules to secrete hydrogen ions and to acidify urine. The ability to acidify urine increases to adult values by about one to two months of age.[25,29] The neonate's decreased renal capacity to reabsorb bicarbonate and excrete hydrogen ions results in lower normal values for serum bicarbonate and blood pH. In addition, the neonate is less able to handle an acid load or to compensate for acid–base abnormalities.

It should be noted that for multiple reasons, the full-term newborn is in a state of metabolic acidosis immediately after birth (arterial pH 7.11–7.36). The blood pH increases to more normal values within 24 hours, mostly due to increased excretion of carbon dioxide via the lungs.[25]

Calcium

Total serum calcium—normal range[12]*:*
neonates 3–24 hr: 9–10.6 mg/dL (2.25–2.65 mmol/L)
neonates 24–48 hr: 7–12 mg/dL (1.75–3 mmol/L)
neonates 4–7 days: 9–10.9 mg/dL (2.25–2.73 mmol/L)
children: 8.8–10.8 mg/dL (2.2–2.7 mmol/L)
adolescents: 8.4–10.2 mg/dL (2.1–2.55 mmol/L)
adults: 8.7–10.2 mg/dL (2.2–2.6 mmol/L)

Ionized calcium—normal range:
neonates 3–24 hr: 4.3–5.1 mg/dL (1.08–1.28 mmol/L)
neonates 24–48 hr: 4–4.7 mg/dL (1–1.18 mmol/L)
infants, children, and adolescents: 4.5–4.92 mg/dL (1.13–1.23 mmol/L)
adults: 4.5–5.3 mg/dL or 1.13–1.33 mmol/L

Calcium plays an integral role in many physiologic functions including muscle contraction, neuromuscular transmission, blood coagulation, bone metabolism, and regulation of endocrine functions. The great majority of calcium in the body (99%) is found in the bone, primarily as hydroxyapatite. Because of the growth that occurs during infancy and childhood, bone mass increases faster than body weight.[30] This increase in bone mass requires a significant increase in total body calcium. The increased calcium requirement is reflected in the higher recommended daily allowances (per kg body weight) in pediatric patients compared to adults.

Calcium regulation in the body has two main goals.[30] First, serum calcium must be tightly regulated to permit the normal physiologic functions in which calcium plays a role. Second, calcium intake must be adequate to permit appropriate bone mineralization and skeletal growth. It is important to remember that bone mineralization may be sacrificed (i.e., calcium may be released from the bone) to allow maintenance of a normal serum calcium concentration.

As in adults, serum calcium in pediatric patients is regulated by a complex hormonal system that involves vitamin D, serum phosphate, parathyroid hormone (PTH), and calcitonin. Briefly, calcium is absorbed in the GI tract, primarily via the duodenum and jejunum.[30] Although some passive calcium absorption occurs when dietary intake is high, most GI absorption of calcium occurs via active transport that is stimulated by 1,25-dihydroxyvitamin D. This occurs especially when dietary intake is low. Calcium excretion is controlled by the kidneys and influenced by multiple hormonal mediators (e.g., PTH, 1,25-dihydroxyvitamin D, and calcitonin). In the mature kidneys, approximately 99% of filtered calcium is reabsorbed by the tubules with the majority (>50%) absorbed by the proximal tubules. Calcium also is absorbed in the loop of Henle, distal tubule, and collecting ducts.

During the first week of life, urinary calcium excretion is inversely related to GA (i.e., more premature infants will have a greater urinary calcium excretion).[25] Compared to adults, urinary calcium excretion is higher in neonates and preterm infants. The urinary calcium-to-creatinine ratio is 0.11 in adults but may be >2 in premature neonates and ranges from 0.05–1.2 in full-term neonates during the first week of life. This high rate of calcium excretion may be related to the immaturity of the renal tubules and may contribute (along with other factors) to neonatal hypocalcemia. In addition, certain medications that are commonly administered to neonates and premature infants, such as furosemide, dexamethasone, and methylxanthines, further increase urinary calcium excretion. These medications may also increase the risk for hypocalcemia as well as nephrocalcinosis and nephrolithiasis.[25]

Measurement of Calcium

Total serum calcium measures all three forms of extracellular calcium: complex bound, protein bound, and ionized. However, ionized calcium is the physiologically active form. Usually a parallel relationship exists between the ionized and total serum calcium concentrations. However, in patients with alterations in acid–base balance or serum proteins, the ionized serum calcium and total serum calcium are affected, respectively, and measurements of total serum calcium may no longer reflect the ionized serum concentration. Neonates have lower serum concentrations of protein (including albumin) and may be acidotic. This results in a lower total serum calcium concentration for a given ionized plasma concentration.[27] Although equations exist to adjust total serum calcium measurements for low concentrations of serum albumin, these equations have limitations and may not be precise. Therefore, ionized calcium should be measured in neonates (if micro-techniques are available) and other pediatric patients with hypoalbuminemia or acid–base disorders.

Hypocalcemia

As in adults, *hypocalcemia* may occur in pediatric patients due to a variety of causes including inadequate calcium intake, hypoparathyroidism, vitamin D deficiency, renal failure, redistribution of plasma calcium (e.g., hyperphosphatemia and citrated blood transfusions), and hypomagnesemia. Hypocalcemia may also occur due to lack of organ response to PTH (e.g., pseudohypoparathyroidism) and in the neonate due to other specific causes. The exact mechanism of how hypomagnesemia causes hypocalcemia is not clearly delineated. Magnesium can affect calcium balance and significant hypomagnesemia can result in hypocalcemia due to intracellular cationic shifts. It is also thought that hypomagnesemia impairs the release of PTH and induces resistance to PTH effects. Because hypomagnesemia can result in hypocalcemia, a serum magnesium concentration is generally obtained in patients with hypocalcemia.

In the pediatric population, hypocalcemia most commonly occurs in neonates. *Early neonatal hypocalcemia* occurs during the first 72 hours of life and may be due to several factors. During fetal development, a transplacental active transport process maintains a higher calcium concentration in the fetus compared to the mother. After birth, this transplacental process suddenly stops. Serum calcium concentrations then decrease, even in healthy full-term newborns, reaching a nadir at 24 hours.[30] The high serum calcium concentrations in utero may also suppress the fetus' parathyroid gland. Thus, early neonatal hypocalcemia may also be due to a relative hypoparathyroidism in the newborn. In addition, newborns may have a decreased response to PTH. Early neonatal hypocalcemia is more likely to occur in premature and low birth weight newborns. It also occurs more commonly in infants of diabetic mothers, infants with intrauterine growth retardation, and in newborns that have undergone prolonged difficult deliveries. Inadequate calcium intake in critically ill newborns also contributes to hypocalcemia.

Late neonatal hypocalcemia, which usually presents during the first 5–10 days of life, is caused by a high phosphate intake. It is much less common than early neonatal hypocalcemia, especially because the phosphorus content of infant formulas was decreased. It may, however, still occur if neonates are inappropriately given whole cow's milk. Cow's milk has a high phosphate load, which can cause hyperphosphatemia and secondary hypocalcemia in the neonate.

Hypocalcemia may also occur in neonates born to mothers with hypercalcemia. The maternal hypercalcemia is usually due to hyperparathyroidism. In utero suppression of the fetal parathyroid gland can lead to hypoparathyroidism and hypocalcemia in the neonate. Hypocalcemia due to inadequate dietary calcium intake rarely occurs in the United States but can occur if infant formula or breast milk is replaced with liquids that contain lower amounts of calcium. Hypocalcemia may be iatrogenically induced if inadequate amounts of calcium are administered in hyperalimentation solutions. Adequate amounts of calcium and phosphorus may be difficult to deliver to preterm neonates due to their high daily requirements and limitations of calcium and phosphorus solubility in hyperalimentation solutions. Certain pediatric malabsorption disorders, such as celiac disease, may also cause inadequate absorption of calcium and vitamin D.

Hypoparathyroidism can be caused by many genetically inherited disorders, such as the DiGeorge syndrome, X-linked hypoparathyroidism, or PTH gene mutations.[30] These and other syndromes must be considered when pediatric patients present with hypoparathyroidism.

In pediatric patients with vitamin D deficiency, hypocalcemia occurs primarily due to decreased intestinal absorption of calcium. The lower amounts of calcium in the blood stimulate the release of PTH from the parathyroid gland. PTH then prevents significant hypocalcemia via several different mechanisms. It causes bone to release calcium, increases urinary calcium reabsorption, and increases the activity of 1α-hydroxylase in the kidneys (the enzyme that converts 25-hydroxyvitamin D into 1, 25-dihydroxyvitamin D, the active form of vitamin D). Hypocalcemia only develops after these compensatory mechanisms fail. In fact, most children with vitamin D deficiency present with rickets before they develop hypocalcemia.[30] In addition to elevated PTH concentrations, children with vitamin D deficiency will have an elevated serum alkaline phosphatase concentration (due to increased osteoclast activity) and a low serum phosphorus (secondary to decreased intestinal absorption and decreased reabsorption in the kidneys), all due to the effects of PTH.

Vitamin D deficiency may be due to several factors including inadequate intake, lack of exposure to sunlight, malabsorption, or increased metabolism of vitamin D (e.g., from medications such as phenobarbital and phenytoin). Generally, patients may have more than one of these factors. For example, institutionalized children (who are not exposed to sunlight) receiving chronic anticonvulsant therapy may be at a greater risk for developing vitamin D deficiency and rickets. Vitamin D deficiency may also occur with liver disease (failure to form 25-hydroxyvitamin D in the liver) and with renal failure (failure to form the active moiety, 1,25-dihydroxyvitamin D, due to a loss of activity of 1α-hydroxylase in the kidneys).

Genetic disorders, such as vitamin D-dependent rickets, may also cause hypocalcemia. The absence of the enzyme, 1-α-hydroxylase, in the kidneys occurs in children with vitamin D-dependent rickets type 1. Therefore, these children cannot convert 25-hydroxyvitamin D to its active form. Children with vitamin D-dependent rickets type 2 have a defective vitamin D receptor, which prevents the normal response to 1,25-dihydroxyvitamin D.[30] (**Minicase 2**.)

Hypocalcemia also occurs when patients receive citrated blood transfusions or exchange transfusions (citrate is used to anticoagulate blood). Citrate forms a complex with calcium and decreases the ionized calcium concentration. This may result in symptoms of hypocalcemia. Pediatric patients at highest risk include those receiving multiple blood transfusions or exchange transfusions, such as neonates treated for hyperbilirubinemia and older children treated for sickle cell crisis. It should be noted that the total serum calcium concentration in these patients can be normal or even elevated, because the calcium-citrate complex is included in the measurement.[30]

Hypercalcemia

Hypercalcemia is an uncommon pediatric electrolyte disorder. As in adults, it may be caused by excess PTH, excess vitamin D, excess calcium intake, excess renal reabsorption of calcium, increased calcium released from the bone, and miscellaneous factors, such as hypophosphatemia or adrenal insufficiency.[30] Causes of hypercalcemia that are of particular interest in pediatric patients include neonatal hyperparathyroidism, hypervitaminosis D, excessive calcium intake, malignancy associated hypercalcemia, and immobilization. Also, several genetic syndromes and disorders may cause hypercalcemia.

Neonatal hyperparathyroidism, an autosomal recessive disorder, can be severe and life-threatening.[30] Typically, these patients have defective calcium sensing receptors in the parathyroid gland. Normally, high serum calcium concentrations would be sensed by the parathyroid gland, and PTH levels would then decrease. In these patients, however, the parathyroid gland cannot sense the high serum calcium concentrations, and PTH continues to be released. This further increases serum calcium concentrations. Transient secondary neonatal hyperparathyroidism occurs in neonates born to mothers with hypocalcemia. Maternal hypocalcemia leads to hypocalcemia in the fetus with secondary hyperparathyroidism. These neonates may be born with skeletal demineralization and bone fractures. Hypercalcemia in these patients usually takes days to weeks to resolve.

MINICASE 2

Rickets in a Child

Raymond D., a 10-year-old male, is admitted to the emergency department from a local pediatric long-term care facility with c/o pain, tenderness, and decreased movement to his right leg. He sustained a fall at the long-term care facility when he was being moved from his bed to his wheel chair. Born at term, he suffered a traumatic birth with severe perinatal asphyxia. He subsequently developed seizures that were controlled by the combined anticonvulsant therapy of phenobarbital and phenytoin. As a result of his asphyxia at birth, he developed spastic cerebral palsy and severe neurodevelopmental delay. He was transferred to the long-term care facility at four months of age and has remained on phenobarbital and phenytoin since that time. Two years ago, he was diagnosed with gastroesophageal reflux disease, which has been controlled with antacids. Medications include phenobarbital elixir 60 mg (15 mL) PO BID; phenytoin suspension 75 mg (3 mL) PO BID; and aluminum hydroxide suspension 10 mL PO QID.

His vital signs include BP 102/70 mm Hg; HR 92 beats/min; RR 24 breaths/min; and temperature 98.6 °F. His height is 125 cm (<3rd percentile for age), and weight is 24 kg (<5th percentile for age). His physical exam of his chest is significant for a pigeon breast deformity and slightly palpable enlargement of costochondral junctions. He has redness in his right leg, 10 cm below the knee, and pain on movement. The preliminary x-ray findings reveal a fracture of his right tibia with osteomalacia and bone changes consistent with rickets.

Significant laboratory data include calcium 8.2 mg/dL (normal for children: 8.8–10.8 mg/dL); ionized calcium: 4 mg/dL (normal for infants to adults: 4.5–4.92 mg/dL); phosphorus: 2.5 mg/dL (normal for 4–11 years: 3.7–5.6 mg/dL); magnesium: 1.6 mg/dL (normal for 2–14 years: 1.5–2.3 mg/dL); albumin 2.9 g/dL (normal for children 7–19 years: 3.7–5.6 g/dL); ALT: 55 units/L (normal for 1–19 years: 5–45 units/L); AST: 65 units/L (normal for children 10–15 years: 10–40 units/L); alkaline phosphatase: 863 units/L (normal for children 2–10 years: 100–320 units/L).

QUESTION: What evidence exists that this patient has rickets? How did his medications affect his serum phosphorus, calcium, and liver enzymes, and how would you modify his drug therapy?

DISCUSSION: Rickets is diagnosed by both radiologic and chemical findings. The preliminary x-ray findings and the physical findings of the pigeon breast deformity (i.e., the sternum and adjacent cartilage appear to be projected forward) and the palpable enlargement of costochondral junctions (rachitic rosary sign) are compatible with the diagnosis of rickets. Serum calcium may be low or normal in patients with rickets, depending on the etiology. The primary causes of rickets in the United States are vitamin D deficiency (with secondary hyperparathyroidism), primary phosphate deficiency, and end-organ resistance to 1,25-dihydroxyvitamin D. In patients with vitamin D deficiency, serum calcium concentrations can be normal or low, phosphorus concentrations are usually low, and alkaline phosphatase activity is elevated. In patients with primary phosphate deficiency, serum calcium is normal, serum phosphorus is low, and alkaline phosphatase is elevated. In patients with end-organ resistance to 1,25-dihydroxyvitamin D, serum calcium is low, serum phosphorus may be low or normal, and serum alkaline phosphatase is elevated.

In this patient, ionized calcium and serum phosphorus are both low and serum alkaline phosphatase is high, all of which are consistent with a diagnosis of rickets. Serum magnesium is normal for age; ALT and AST are slightly elevated. The serum magnesium was obtained because hypomagnesemia may also cause hypocalcemia. He also has hypoalbuminemia. A total serum calcium measures all three forms of extracellular calcium: complex bound, protein bound, and ionized. In patients with low albumin, the concentration of ionized calcium will be increased for a given total serum calcium concentration. Equations can be used to “correct” total serum calcium measurements for low concentrations of serum albumin, but these equations have limitations and may not be precise. Thus, in patients with low albumin (like this patient), an ionized serum calcium should be obtained.

His medications affected his laboratory tests. He is receiving an aluminum-containing antacid, which binds phosphorus in the GI tract. This resulted in decreased absorption of phosphorus and contributed to his low serum phosphorus. Enzyme-inducing anticonvulsants, such as phenobarbital and phenytoin, will increase the metabolism of vitamin D and may result in a deficiency of vitamin D with resultant anticonvulsant-induced osteomalacia and rickets. Both the aluminum-containing antacid and the anticonvulsants contributed to him developing rickets, and thus, to the elevated serum alkaline phosphatase. In addition, due to his other medical conditions, he is nonambulatory and resides at a long-term care facility. Thus, he may have a lack of exposure to sunlight and, therefore, a lack of vitamin D. This lack of vitamin D also would contribute to the development of rickets.

For treatment of his rickets, he should be started on oral supplements of calcium, phosphorous, and vitamin D. However, modifications in his preadmission medications should be made. The aluminum-containing antacid (aluminum hydroxide suspension) should be discontinued and replaced with a calcium-containing antacid (e.g., calcium carbonate). The amount of calcium in this new antacid should then be subtracted from any calcium supplement that would be started in the hospital, so that the total daily dose of calcium stays the same. Alternatively, the total dose of calcium supplement can be given as calcium carbonate. Discontinuing the aluminum-containing antacid will result in a greater amount of phosphorus absorbed enterally. This will then require a decrease in the oral supplement of phosphate (depending on serum phosphorus concentrations). Once he is stable, his neurologist should be consulted to see if other anticonvulsants that have less of an enzyme-inducing effect could be used to treat his seizures.

Excessive intake of vitamin D or calcium may also cause hypercalcemia. Typically, this may occur in children who are being treated with vitamin D and calcium with excessive doses. Excess calcium in hyperalimentation solutions commonly results in hypercalcemia.

Compared to adults, hypercalcemia from immobilization occurs more frequently in children, especially adolescents.[30] This is due to a higher rate of bone remodeling in these patients. Immobilization of children and adolescents may be required due to specific injuries such as leg fractures, spinal cord paralysis, burns, or other severe medical conditions. In children with leg fractures requiring traction, hypercalcemia usually occurs within one to three weeks. Immobilization may also result in isolated hypercalciuria, which may result in nephrocalcinosis, kidney stones, or renal insufficiency.

Phosphorus

Normal range[23]*:*
neonates 0–5 days: 4.8–8.2 mg/dL (1.55–2.65 mmol/L)
children 1–3 yr: 3.8–6.5 mg/dL (1.23–2.1 mmol/L)
children 4–11 yr: 3.7–5.6 mg/dL (1.2–1.8 mmol/L)
adolescents 12–15 yr: 2.9–5.4 mg/dL (0.94–1.74 mmol/L)
adolescents 16–19 yr: 2.7–4.7 mg/dL (0.87–1.52 mmol/L)
adults: 2.3–4.7 mg/dL (0.74–1.52 mmol/L)

Phosphorus is the primary intracellular anion and plays an integral role in cellular energy and intracellular metabolism. It is also a component of phospholipid membranes and other cell structures. The great majority of phosphorus in the body (85%) is found in the bone, while <1% of phosphorus is found in the plasma. Like calcium, phosphorus is essential for bone mineralization and skeletal growth. During infancy and childhood, a positive phosphorus balance is required for proper growth to allow adequate amounts of phosphorus to be incorporated into bone and new cells. The higher phosphorus requirement that is needed to facilitate growth may help explain the higher serum concentrations seen in the pediatric population compared to adults.

The kidney is the primary organ that regulates phosphorus balance. Approximately 90% of plasma phosphate is filtered by the glomerulus with the majority being actively reabsorbed at the proximal tubule. Some reabsorption also occurs more distally, but phosphate is not significantly secreted along the nephron.[23] Unlike other active transport systems, phosphate reabsorption, both proximal and distal, is greater in the neonatal kidney compared to adults.[25,29] Thus, the neonatal kidney tends to retain phosphate, perhaps as a physiologic adaptation to the high demands for phosphate that are required for growth. Neonatal renal phosphate reabsorption may be regulated by growth hormone.[29]

Hypophosphatemia

As in adults, *hypophosphatemia* may occur in pediatric patients due to several causes including increased renal excretion, decreased phosphate or vitamin D intake, or intracellular shifting. Causes of excessive renal phosphorus excretion in pediatric patients include hyperparathyroidism, metabolic acidosis, diuretics, glucocorticoids, glycosuria, IV fluids and volume expansion, kidney transplantation, and inherited disorders such as hypophosphatemic rickets.

Inadequate dietary phosphate intake is an unusual cause of hypophosphatemia in adults. However, infants are more predisposed to nutritional hypophosphatemia due to their higher phosphorus requirements.[23] The phosphorus requirements of premature infants are even higher due to their rapid skeletal growth. If premature infants are fed regular infant formula (instead of premature infant formula that contains additional calcium and phosphorus), phosphorus deficiency and rickets may occur. Phosphorous deficiency and rickets also can occur in pediatric patients who receive aluminum hydroxide containing antacids, which bind dietary and secreted phosphorous and prevent its absorption from the GI tract. Inadequate vitamin D intake and genetic causes of vitamin D deficiency (e.g., vitamin D-dependent rickets type 1) also can result in hypophosphatemia in pediatric patients.

Hypophosphatemia due to intracellular shifting of phosphorus occurs with processes that stimulate intracellular phosphorus utilization. For example, high serum levels of glucose will stimulate insulin. Insulin then enables glucose and phosphorus to move into the cell, where phosphorus is used during glycolysis. Intracellular shifting of phosphorus also occurs during anabolism, for example, in patients receiving hyperalimentation and during refeeding in those with protein-calorie malnutrition (e.g., severe anorexia nervosa). The high anabolic (growth) rate in infants (especially premature infants) and children make them more susceptible to hypophosphatemia when adequate amounts of phosphate are not supplied in the hyperalimentation solution. Hypophosphatemia, due to refeeding malnourished children, usually occurs within five days of refeeding. It may be prevented by a more gradual increase in nutrition and with phosphate supplementation.[23]

Hyperphosphatemia

Hyperphosphatemia in pediatric patients may be caused by decreased excretion of phosphorus, increased intake of phosphate or vitamin D, or a shift of intracellular phosphate to extracellular fluid. The most common cause of hyperphosphatemia in the pediatric population is decreased excretion of phosphorus due to renal failure. Excessive phosphorus intake in pediatric patients (especially in those with renal dysfunction or in neonates whose renal function is normally decreased due to immaturity) is a common cause of hyperphosphatemia.[23] Hyperphosphatemia may also occur if neonates are inappropriately given whole cow's milk. As previously mentioned, cow's milk contains a high phosphate load, which can cause hyperphosphatemia and secondary hypocalcemia in the neonate. Administration of sodium phosphorus laxatives or enemas to infants and children may also result in excessive phosphate intake. In addition, the pediatric dosing of phosphate supplements may be confusing to some due to the multiple salts available and multiple units of measure. This may result in unintentional overdoses with resultant hyperphosphatemia.

Magnesium

Normal range[12]*:*
neonates 0–6 days: 1.2–2.6 mg/dL (0.49–1.07 mmol/L)
neonates 7 days to children 2 yr: 1.6–2.6 mg/dL (0.66–1.07 mmol/L)
children 2 yr to adolescents 14 yr: 1.5–2.3 mg/dL (0.62–0.95 mmol/L)
adults: 1.3–2.1 mEq/L (0.65–1.05 mmol/L)

Magnesium plays an important role in neuromuscular function and is a required cofactor for many enzymatic systems in the body. Approximately 50% of magnesium is located in bone with one third being slowly exchangeable with extracellular fluid. About 45% of magnesium is found in the intracellular fluid with only 5% in extracellular fluid. The kidney is the primary organ responsible for magnesium excretion. Approximately 95–97% of filtered magnesium is reabsorbed; 15% in the proximal tubule, 70% in the thick ascending limb of Henle, and 5–10% in the distal tubule.[23] In the neonate, reabsorption of magnesium may be increased in the proximal tubule. Thus, the immature neonatal kidney tends to retain magnesium compared to adults.[25] This results in slightly higher normal values for serum magnesium in neonates and infants compared to older children and adults. In fact, serum magnesium concentrations in the newborn have been shown to be inversely related to GA at birth and PMA. In other words, more immature neonates will have slightly higher serum magnesium concentrations.[31,32]

Hypomagnesemia

Hypomagnesemia occurs in pediatric patients due to excessive renal or GI losses, decreased GI absorption, decreased intake, and specific neonatal causes.[23] Hypomagnesemia may occur in neonates due to several maternal causes. Maternal diuretic use, laxative overuse or abuse, diabetes mellitus, or decreased intake due to vomiting during pregnancy may cause maternal hypomagnesemia and lead to hypomagnesemia in the newborn.[33] Hypomagnesemia also commonly occurs in neonates with intrauterine growth retardation (due to deficient placental transfer of magnesium) and in neonates who receive exchange transfusions with citrated blood.

Excessive renal losses of magnesium may be due to a variety of reasons. Of particular pediatric concern is the use of medications (e.g., diuretics, amphotericin, and cisplatin) that may cause magnesium wasting. Hypomagnesemia may also occur due to rare hereditary renal magnesium-losing syndromes, such as Bartter syndrome and autosomal recessive renal magnesium–wasting syndrome. Excessive GI losses of magnesium may occur in pediatric patients with diarrhea or large losses of gastric contents (e.g., emesis or nasogastric suction). Decreased GI absorption of magnesium may occur in patients with short gut syndrome. These patients have had a portion of their small bowel removed, which results in poor intestinal absorption. Other important pediatric GI diseases that may result in hypomagnesemia include cystic fibrosis, inflammatory bowel disease, and celiac disease.[23]

Poor magnesium intake may also result in hypomagnesemia. Although this rarely occurs in children fed orally, it may occur in hospitalized children receiving inadequate amounts of magnesium in IV fluids or hyperalimentation. Hypomagnesemia also can occur during the refeeding of children with protein-calorie malnutrition (e.g., severe anorexia nervosa). These patients have low magnesium reserves but a high requirement of magnesium because of cellular growth.[23]

Hypermagnesemia

As in adults, the most common cause of *hypermagnesemia* in pediatric patients is renal dysfunction. However, in neonates, the most common cause is the IV infusion of magnesium sulfate in the mother for the treatment of preeclampsia or eclampsia.[23,33] The high levels of magnesium in the mother are delivered transplacentally to the fetus. Neonates and young infants are also more prone to hypermagnesemia, due to their immature renal function. Thus, these patients cannot easily tolerate a magnesium load. Other common pediatric causes of hypermagnesemia include excessive intake due to magnesium-containing antacids, laxatives, or enemas.

AGE-RELATED DIFFERENCES IN KIDNEY FUNCTION TESTS

Serum Creatinine

Jaffe Method—normal range[34]*:*
neonates: 0.3–1 mg/dL (27–88 μmol/L)
infants: 0.2–0.4 mg/dL (18–35 μmol/L)
children: 0.3–0.7 mg/dL (27–62 μmol/L)
adolescents: 0.5–1 mg/dL (44–88 μmol/L)
adult males: 0.6–1.2 mg/dL (53–106 μmol/L)
adult females: 0.5–1.1 mg/dL (44–97 μmol/L)

Isotope Dilution Mass Spectrometry (IDMS)-Traceable Enzymatic Method—normal range[12]*:*
neonates to children 4 yr: 0.03–0.5 mg/dL (2.65–44.2 μmol/L)
children 4–7 yr: 0.03–0.59 mg/dL (2.65–52.2 μmol/L)
children 7–10 yr: 0.22–0.59 mg/dL (19.4–52.2 μmol/L)
children and adolescents 10–14 yr: 0.31–0.88 mg/dL (27.4–77.8 μmol/L)
adolescents >14 yr: 0.5–1.06 mg/dL (44.2–93.7 μmol/L)

Serum creatinine (SCr) is a useful indicator of renal function and can be used to estimate GFR. Creatinine is generated from the metabolism of creatine and creatine phosphate, a high-energy biochemical important in muscle activity. Creatinine is produced in muscles, released into the extracellular fluid, and excreted by the kidneys. Excretion of creatinine is primarily via glomerular filtration, but a smaller amount undergoes tubular secretion. The amount of creatinine that is secreted by the tubules increases in patients as GFR decreases. Thus, creatinine clearance (CrCl) will overestimate the actual GFR in patients with renal insufficiency.[35]

In pediatric patients, three major factors influence the SCr concentration: the patient's muscle mass per unit of body size, their GFR, and (in newborns) the exogenous (maternal) creatinine load.[36] At birth, the newborn's SCr reflects the maternal SCr. Because SCr crosses the placenta, if a pregnant woman has an elevated SCr, then the concentration of creatinine in the fetus also will be elevated. In fact, the plasma creatinine concentration of umbilical cord blood is almost equal to the creatinine concentration in the mother.[27] In full-term newborns, SCr may increase slightly, shortly after birth, due to the contraction of the ECW compartment.[36] SCr then decreases over the first few days of life and usually reaches 0.4 mg/dL (Jaffe method) by about 10 days of age.[25,36] The apparent half-life of this postnatal decrease in SCr is about 2.1 days in normal full-term infants and is due to the ongoing maturation of the kidneys and progressive increase in GFR. SCr is higher at birth in premature newborns compared to full-term newborns, and the postnatal decrease in SCr may occur more slowly. This is due to the preterm newborn's more immature kidneys and lower GFR.[27,37]

Compared to adults, pediatric patients have a lower muscle mass per unit of body size. Because the production of creatinine is dependent on muscle mass, this results in significantly lower normal values for SCr for neonates, infants, and children. The percentage of muscle mass differs with various pediatric age groups and increases with age from birth through young adulthood.[36] This increase in muscle mass accounts for the increase in the normal values for SCr with increasing age (see normal values for SCr above).

Creatinine excretion is dependent on GFR and, as in adults, SCr will become elevated in pediatric patients with renal dysfunction. For example, an infant with a SCr as measured by the Jaffe method of 0.8 mg/dL (twice the normal value for age) will have approximately a 50% decrease in GFR. Using the age-appropriate normal values to interpret SCr is essential. In the above example, a SCr of 0.8 mg/dL (which would be considered normal in an adult) denotes significant renal dysfunction in younger patients. Correct interpretation of SCr values is extremely important because the doses of fluids, many electrolytes, and medications that are renally eliminated will need to be adjusted. Misinterpretation of SCr (e.g., not recognizing renal dysfunction) can result in serious and potentially fatal fluid and electrolyte imbalances and overdosing of medications.

Reliable and accurate measurement of SCr is clinically important to properly assess renal function. Historically, SCr was measured by the alkaline picrate-based method, also known as the *Jaffe method*. However, substances that interfere with the measurement of creatinine by this method (i.e., noncreatinine chromogens such as uric acid, glucose, fructose, and acetone) can cause an overestimation of SCr and, thus, an underestimation of kidney function. In addition, certain medications and endogenous substances (e.g., bilirubin, lipemia, and hemolysis) may interfere with the determination of SCr by this method.[6,11] This interference may be a problem in the neonatal population because neonates often have hyperbilirubinemia or lipemia, and blood sampling in neonates often results in hemolysis.

Inaccuracies of the Jaffe method and other methodologies, have lead the National Kidney Disease Education Program to recommend a recalibration and standardization of SCr measurements.[38] This has resulted in implementation of improved methods of SCr determinations, such as an enzymatic assay with an IDMS-traceable international standard. It is important to know what methodology your laboratory is using, because measurement by newer assays will result in lower SCr determinations. Thus, the normal value of SCr for a specific patient will depend on the assay method being used and the age of the patient (see normal values for SCr above). Further information about laboratory measurement and reporting of SCr can be found in Chapter 11.

Age-Related Physiologic Development of Renal Function

Compared to adults, a newborn's kidneys are anatomically and functionally immature. The primary functions of the kidney (glomerular filtration, tubular secretion, and tubular reabsorption) are all decreased in the full-term newborn. These renal functions are even further decreased in the premature infant. After birth, glomerular and tubular renal function increase (i.e., mature) with PNA. During the first two years of life, kidney function matures to adult levels and in the following order: (1) glomerular filtration, (2) tubular secretion, and (3) tubular reabsorption. The interpretation of pediatric kidney functions tests can be better understood if one knows how each function of the kidneys matures during the first two years.

Glomerular filtration. In the fetus, nephrogenesis (i.e., the formation of new nephrons) begins at 8 weeks of gestation and continues until about 36 weeks of gestation.[39] Although the number of adult nephrons (~1 million) is reached at this time, the nephrons are smaller and not as functionally mature as the nephrons found in an adult kidney.[24] After 36 weeks of gestation, no new nephrons are formed. However, renal mass continues to increase due to the increase in renal tubular growth. GFR is very low in the young fetus but gradually increases during gestation (**Figure 21-4**). Before 36 weeks of gestation,

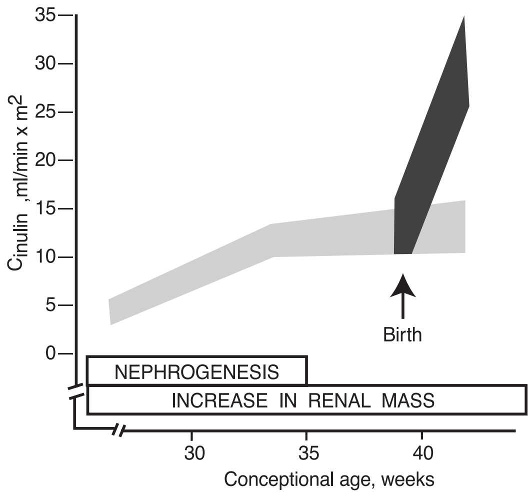

FIGURE 21-4. Maturation of GFR in relation to conceptional age. (Reproduced, with permission, from Guignard JP. The neonatal stressed kidney. In: Gruskin AB, Norman ME, eds. Pediatric nephrology. Philadelphia: Martinus Nijhoff Publishers; 1981:507.)

the increase in GFR is primarily due to nephrogenesis and the increase in the number of new glomeruli. From 36 weeks of gestation until birth, a much smaller increase in GFR occurs as renal mass and kidney function increase.[40]

At birth, GFR increases dramatically compared to what it was in utero (Figure 21-4). This dramatic increase in GFR, which occurs at birth and continues during the early postnatal period, is due to several important hemodynamic and physiologic changes. Cardiac output and systemic blood pressure increase at birth and a significant decrease in renal vascular resistance occurs. These changes result in an increase in renal blood flow and effective glomerular filtration pressure. In addition, alterations in the pattern of renal blood flow distribution occur and the permeability of the glomerular membrane and surface area available for filtration increase.[24,39,40] All of these changes help to increase GFR.

Despite the increase in GFR that occurs during this time, GFR is still very much decreased in comparison to adults. As determined by creatinine or inulin clearance, the GFR in a full-term newborn is only 10–15 mL/min/m^2 (2–4 mL/min). GFR then doubles by one to two weeks of age to 20–30 mL/min/m^2 (8–20 mL/min).[39] Adult values of GFR are approached by about 6–12 months of age (70–90 mL/min/m^2). Compared to full-term newborns, GFR in premature newborns is much lower at birth (5–10 mL/min/m^2 or 0.7–2 mL/min) and increases at a less dramatic rate during the first one to two weeks after birth (10–12 mL/min/m^2 or 2–4 mL/min).[39] After the first postnatal week, the rate of increase in GFR is comparable in preterm and full-term infants, but the actual GFR value is still lower in preterm infants than in full-term infants.

Renal tubular function. Tubular secretion and reabsorption are both decreased in the full-term newborn. This is due to the small size and mass of the renal tubules, decreased peritubular blood flow, and immature biochemical processes that supply energy for active transport. In addition, full-term newborns have a limited ability to concentrate urine and have lower urinary pH values.[39] In the preterm newborn, renal tubular functions are further decreased. Limitations of the newborn's tubular function with respect to the renal handling of serum electrolytes are listed above within the discussion section of each serum electrolyte.

Tubular secretion transports certain electrolytes and medications from the peritubular capillaries into the lumen of the renal tubule. At birth, tubular secretion is only 20–30% of adult values and slowly matures by about eight months of age. Tubular reabsorption, which also is decreased at birth, may not fully mature until one to two years of age. Thus, during infancy, a glomerulotubular imbalance occurs, with GFR maturing at a faster rate than renal tubular function.

The decreased renal function in newborns and the maturational changes in GFR and tubular function that occur throughout early infancy have important implications for the interpretation of laboratory data. For example, one must remember that even with a normal SCr for age, neonates and infants still have decreased renal function compared to adults. This decreased renal function must be taken into account, especially in the very young, when dosing electrolytes or medications that are eliminated by the kidneys. In addition, as in adults, certain medications, diseases, and medical conditions (such as hypoxic events that may occur in newborns) may cause further decreases in renal function.

Standardization of Creatinine Clearance

Creatinine clearance (CrCl) can be expressed using several different units of measure, including mL/min, mL/min/m^2, or mL/min/1.73 m^2. To better compare the CrCl of patients of different body sizes, CrCl is most commonly standardized to the BSA of an average-sized adult (1.73 m^2). Thus, CrCl is most commonly measured as mL/min/1.73 m^2. Using these units is especially helpful in pediatric patients where a large range of body sizes occurs. For example, the average BSA ranges from 0.25 m^2 in a full-term newborn to 1.34 m^2 in a 12-year-old.[1] Expressing CrCl in mL/min or even mL/min/m^2 over this wide of a range of BSAs would give an extremely wide range of values. (**Minicase 3.)**

Estimating Body Surface Area in Pediatric Patients

Body surface area (BSA) can be estimated using several different methods. In pediatrics, BSA is most commonly estimated using standard nomograms or equations and the patient's measured height and weight.[1] Two equations are commonly used in pediatrics, an older equation (the DuBois formula)[41]:

$$\text{BSA (m}^2\text{)} = \text{Wt (kg)}^{0.425} \times \text{Ht (cm)}^{0.725} \times 0.007184 \qquad (1)$$

and a more simplified equation[42]

$$\text{BSA (m}^2\text{)} = \text{the square root of ([Ht (cm)} \times \text{Wt (kg)]/3600)} \qquad (2)$$

Estimation of the patient's BSA is required to calculate CrCl from a urinary collection.

Determination of CrCl from a Urinary Creatinine Collection

The same equation that is used in adults can be used in pediatric patients to calculate CrCl from a timed urine collection. The following equation is used:

$$\text{CrCl} = (\text{UV/P}) \times (1.73/\text{BSA}) \qquad (3)$$

where CrCl is in units of mL/min/1.73 m^2; U is the urinary creatinine concentration in mg/dL; V (mL/min) is the total urine volume collected in milliliters divided by the duration of the collection in minutes; P is the SCr concentration in mg/dL; and BSA is the patient's BSA in m^2.

Ideally, urine should be collected over a 24-hour period. However, a full 24-hour collection period is very difficult in pediatric patients, especially in those who do not have full control over their bladder and do not have a urinary catheter in place. Thus, shorter collection periods (e.g., 8 or 12 hours) are sometimes used. Urinary specimen bags can be placed to collect urine in neonates and infants, but incomplete collection due to leakage of urine often occurs. The incomplete collection of urine will result in an inaccurate calculation of CrCl.

With any urine collection for creatinine determination, it is important to have the patient empty their bladder and discard this specimen before beginning the urine collection. All urine during the time period should be collected, including the urine

MINICASE 3

Vancomycin Dose Determination Based on Estimated Creatinine Clearance in a Child

Morgan R., a four-year-old female, is currently in the PICU recovering from cardiac surgery (postoperative day 7). Her blood culture today is positive for methicillin–resistant *Staphylococcus aureus* and vancomycin therapy is to be initiated. Vital signs include BP 95/62 mm Hg, HR 142 beats/min, RR 27 breaths/min, and temperature 101.2 °F. Her height is 101 cm (50th percentile for age); weight is 16 kg (50th percentile for age); BSA is 0.67 m^2. Laboratory data include sodium 140 mEq/L, potassium 3.8 mEq/L, chloride 102 mEq/L, total CO_2 28 mEq/L, BUN 42 mg/dL, SCr 2.4 mg/dL (Jaffe method), and glucose 109 mg/dL.

QUESTION: Knowing the following information, what dose of vancomycin would you recommend for this patient?

The normal dose of vancomycin used at this hospital for a child of this age is 40 mg/kg/day divided q 6 hr. The recommended dosing adjustment for patients with renal dysfunction is as follows[50]:

- CrCl 70–89 mL/min/1.73 m^2: administer the normal dose q 8 hr
- CrCl 46–69 mL/min/1.73 m^2: administer the normal dose q 12 hr
- CrCl 30–45 mL/min/1.73 m^2: administer the normal dose q 18 hr
- CrCl 15–29 mL/min/1.73 m^2: administer the normal dose q 24 hr

DISCUSSION: Vancomycin is primarily eliminated by glomerular filtration in the kidney; 80–90% of a dose is excreted in the urine as unchanged drug. Her BUN and SCr are elevated indicating that she has renal impairment. Thus, the dose of vancomycin must be adjusted for her renal dysfunction. To recommend an appropriate dose, her CrCl needs to be calculated. Because this laboratory used the Jaffe method to measure SCr, an estimation of CrCl from her SCr and height can be obtained by using equation (5) and the k value of 0.55 for children 2–12 years of age (Table 21-3). Her estimated CrCl is 23 mL/min/1.73 m^2. To determine the appropriate dose of vancomycin, one must first calculate the normal dose (i.e., as if the patient did not have any renal dysfunction), and then adjust the dose according to the given guidelines. Because she weighs 16 kg, the normal dose of vancomycin would be 160 mg q 6 hr (40 mg/kg/day divided q 6 hr). Because her CrCl is 23 mL/min/1.73 m^2, she should receive 160 mg q 24 hr. Appropriate therapeutic drug monitoring should be performed to adjust this initial dosing regimen. It should be noted that if SCr was measured by a newer enzymatic assay using IDMS-based reference standards, then equation (6) should be used to estimate CrCl. If equation (5) and the constants from Table 21-3 were used, it would result in an overestimation of CrCl by approximately 20–30%. Additionally, almost all drug dosage guidelines for patients with renal impairment (including those recommended by pharmaceutical manufacturers) were developed using SCr as measured by older assay methodologies. Thus, estimated CrCl values may not correlate well with these dosage guidelines. These limitations further emphasize the need for appropriate therapeutic drug monitoring and follow-up dosage adjustment.

that would be voided at the end of the collection period. A SCr is usually obtained once during the urinary collection period (ideally at the midpoint) if the patient has stable renal function. If the patient's renal function is changing, then two SCr samples (one at the beginning of the urine collection and one at the end of the urine collection) may be obtained. The average SCr can then be used in the above equation.[3]

Because of the inherent problems of collecting a 24-hour urine sample from pediatric patients and receiving inaccurate calculations, CrCl (or GFR) is often estimated using prediction equations that consider the patient's age, height, gender, and SCr (see below). In fact, using a 24-hour timed urine specimen to calculate CrCl has been shown to be no more reliable (and often less reliable) than using equations based on SCr.[43] Therefore, the National Kidney Foundation recommends that GFR should be estimated in children and adolescents using prediction equations, such as the one by Schwartz.[36] A timed urine collection (e.g., 24-hour sample) may be useful for (1) estimations of GFR in patients with decreased muscle mass (e.g., muscle wasting, malnutrition, or amputation) or in those receiving special diets (e.g., vegetarian diets or creatine supplements); (2) assessments of nutritional status or diets; and (3) evaluations for the need to start dialysis.[43]

Estimating CrCl from Serum Creatinine

Several methods (for example, the Cockcroft-Gault equation) are used in adults to estimate CrCl from a SCr. These equations cannot be used in pediatrics because pediatric patients have a different ratio of muscle mass to SCr. In addition, the amount of pediatric muscle mass per body weight changes over time. Adult equations are based on adult muscle mass and adult urinary creatinine excretion rates. Thus, the use of adult equations in pediatric patients will result in erroneous calculations.

Several predictive equations have been developed to estimate CrCl in pediatric patients. One simple equation was developed for use in children 1–18 years of age with stable SCr[44]:

$$\text{CrCl (mL/min/1.73 m}^2) = 0.48 \times \text{Ht (cm)/SCr (mg/dL)} \quad (4)$$

This equation was found to be clinically useful in predicting CrCl in children. However, it may be less accurate in children with a height <107 cm.[45]

Another equation, developed by Schwartz, is more commonly used to estimate GFR in pediatric patients:

$$\text{GFR (mL/min/1.73 m}^2) = k \times \text{Length (cm)/SCr (mg/dL)} \quad (5)$$

where k is a constant of proportionality.[36] In patients with stable renal function, k is directly related to the muscle component of body weight, which correlates well with the daily rates of

TABLE 21-3. Mean Values of k by Age Group

AGE GROUP	MEAN k VALUE
Low birth weight infants ≤1 yr	0.33
Full-term infants ≤1 yr	0.45
Children 1–12 yr	0.55
Females 13–21 yr	0.55
Males 13–21 yr	0.70

Source: Modified from reference 36.

urinary creatinine excretion. Because the percentage of muscle mass per body weight varies for different age groups, a different value for k must be used for different age groups. In addition to age, the value of k will be affected by body composition; thus, the values for k listed in **Table 21-3** should be used in pediatric patients with average build.

It is important to remember that these two equations were developed using the Jaffe method to assay SCr. Values for SCr as measured by newer methods (e.g., the IDMS-traceable enzymatic method) will be lower than those measured by the Jaffe method (especially at low concentrations of SCr). This will result in an overestimation of GFR if these equations and constants are used when SCr is measured by the newer assays. In fact, use of the Schwartz equation and constants may overestimate GFR by approximately 20–30%.[46] Therefore, revised constants for the Schwartz equation must be determined to better estimate GFR when SCr is measured by newer assays using IDMS-based reference standards.

Recently, an "updated" Schwartz bedside equation, based on SCr measured by a newer enzymatic method, was developed in children 1–16 years of age.[47]

$$\text{GFR (mL/min/1.73 m}^2\text{)} = 0.413 \times \text{Ht (cm)/SCr (mg/dL)} \quad (6)$$

The pediatric patients in which this equation was developed had mild-to-moderate chronic kidney disease (95% of measured GFR values were between 21–76 mL/min/1.73 m^2) and were short in stature for their age and gender. Thus, further studies of this equation in children with higher GFRs and a more normal body habitus are needed before it can be widely applied to the pediatric population.

The above equations may not be accurate in certain pediatric populations, including those patients with unstable renal function, abnormal body habitus (e.g., obesity or malnutrition), decreased muscle mass (e.g., cardiac patients), severe chronic renal failure, or insulin dependent diabetes.[36,48] If clinically indicated, CrCl should be determined by a timed urine collection in these patients.

Estimating GFR from Serum Cystatin C

To improve calculated estimations of GFR, other endogenous markers of renal function have been investigated. Cystatin C is a cysteine protease inhibitor that is produced by all nucleated cells at a relatively constant rate; it is freely filtered by the kidneys. Its rate of production is so constant that serum cystatin C levels are not thought to be influenced by muscle mass, gender, body composition, and age (after 12 months). Reciprocal values of serum cystatin C have been correlated to measured GFR values in adults and children.[46] However, serum cystatin C alone may not accurately estimate GFR in renal transplant patients, patients with high C-reactive protein, diabetes with ketonuria, or thyroid dysfunction. Thus, other equations have been developed which incorporate multiple patient parameters and endogenous markers of renal function. One such pediatric equation includes terms for the ratio of height to SCr, the reciprocals of serum cystatin C and BUN, a factor for gender, and a separate factor for height alone.[47] This complex equation is not clinically friendly but is being further investigated in the National Institutes of Health sponsored Chronic Kidney Disease in Children study. Results of this investigation may yield a more accurate equation to estimate GFR in pediatric patients. Unfortunately at this time, serum cystatin C assays are not readily available at many institutions.

AGE-RELATED DIFFERENCES IN LIVER FUNCTION TESTS

Serum Albumin

Normal range[9]:
neonates 0–5 days, body weight <2.5 kg: 2–3.6 g/dL (20–36 g/L)
neonates 0–5 days, body weight >2.5 kg: 2.6–3.6 g/dL (26–36 g/L)
children 1–3 yr: 3.4–4.2 g/dL (34–42 g/L)
children 4–6 yr: 3.5–5.2 g/dL (35–52 g/L)
children and adolescents 7–19 yr: 3.7–5.6 g/dL (37–56 g/L)
adults: 4–5 g/dL (40–50 g/L)

Serum proteins, including albumin, are synthesized by the liver. Thus, measurements of serum total protein, albumin, and other specific proteins are primarily a test of the liver's synthetic capability. Maturational differences in the liver's ability to synthesize protein help determine the normal range for serum albumin concentrations. The liver of the fetus is able to synthesize albumin beginning at approximately seven to eight weeks of gestation. However, the predominant serum protein in early fetal life is α-fetoprotein. As gestation continues, the concentration of albumin increases, while α-fetoprotein decreases. At approximately three to four months of gestation, the fetal liver is able to produce each of the major serum protein classes. However, serum concentrations are much lower than those found at maturity.[49]

At birth, the newborn liver is anatomically and functionally immature. Because of the immature liver function and a decreased ability to synthesize protein, full-term neonates have decreased concentrations of total plasma proteins, including albumin, γ-globulin, and lipoproteins. Concentrations in premature newborns are even lower, with serum albumin levels as low as 1.8 g/dL.[12] Adult serum concentrations of serum albumin (~3.5 g/dL) are reached only after several months of age. Conditions that cause abnormalities in serum albumin in pediatric patients are the same as in adults and can be reviewed in Chapter 15.

Liver Enzymes

Alanine aminotransferase (ALT, also called SGPT)—normal range[3,12]*:*
neonates 0–7 days: 6–40 units/L (0.1–0.7 μkat/L)
neonates 8–30 days: 8–40 units/L (0.1–0.7 μkat/L)
infants: 12–45 units/L (0.2–0.8 μkat/L)
children and adolescents 1–19 yr: 5–45 units/L (0.1–0.8 μkat/L)
adults: 7–41 units/L (0.1–0.7 μkat/L)

Aspartate aminotransferase (AST, also called SGOT)—normal range:
neonates 0–7 days: 24–100 units/L (0.4–1.7 μkat/L)
neonates 8–30 days: 22–71 units/L (0.4–1.2 μkat/L)
infants: 22–63 units/L (0.4–1.1 μkat/L)
children 1–3 yr: 20–60 units/L (0.3–1 μkat/L)
children 3–9 yr: 15–50 units/L (0.3–0.8 μkat/L)
children and adolescents 10–15 yr: 10–40 units/L (0.2–0.7 μkat/L)
adolescents 16–19 yr: 5–45 units/L (0.1–0.8 μkat/L)
adults: 12–38 units/L (0.2–0.6 μkat/L)

Alkaline phosphatase—normal range:
infants: 150–420 units/L (2.5–7.01 μkat/L)
children 2–10 yr: 100–320 units/L (1.67–5.34 μkat/L)
adolescent males 11–18 yr: 100–390 units/L (1.67–6.51 μkat/L)
adolescent females 11–18 yr: 100–320 units/L (1.67–5.34 μkat/L)
adults: 33–96 units/L (0.55–1.6 μkat/L)

Lactate dehydrogenase—normal range:
neonates 0–4 days: 290–775 units/L (4.84–12.94 μkat/L)
neonates 4–10 days: 545–2000 units/L (9.1–33.4 μkat/L)
infants 10 days–24 mo: 180–430 units/L (3.01–7.18 μkat/L)
children 24 mo–12 yr: 110–295 units/L (1.84–4.93 μkat/L)
adolescents >12 yr: 100–190 units/L (1.67–3.17 μkat/L)
adults: 115–221 units/L (1.92–3.69 μkat/L)

The normal reference ranges for liver enzymes are higher in pediatric patients compared to adults. This may be due to the fact that the liver makes up a larger percentage of total body weight in infants and children compared to adults. For certain enzymes, such as alkaline phosphatase, the higher normal concentrations in childhood represent higher serum concentrations of an isoenzyme from other sources, specifically bone. Approximately 80% of alkaline phosphatase originates from liver and bone. Smaller amounts come from the intestines, kidneys, and placenta. Normally, growing children have higher osteoblastic activity during the bone growth period and an influx into serum of the alkaline phosphatase isoenzyme from bone.[11] Thus, the higher normal concentrations of alkaline phosphatase in childhood primarily represent a higher rate of bone growth. After puberty, the liver is the primary source of serum alkaline phosphatase.

One must keep these age-related differences in mind when interpreting liver enzyme test results. For example, an isolated increase in alkaline phosphatase in a rapidly growing adolescent—whose other liver function tests are normal—would not indicate hepatic or biliary disease, but merely a rapid increase in bone growth. As in adults, increases in AST and ALT in pediatric patients are associated with hepatocellular injury, while elevations of alkaline phosphatase are associated with cholestatic disease. Cholestasis and bone disorders (such as osteomalacia and rickets) are common causes of elevated serum alkaline phosphatase concentrations in pediatric patients.

Bilirubin

Total bilirubin, premature neonates—normal range[3]*:*
0–1 day: <8 mg/dL (<137 μmol/L)
1–2 days: <12 mg/dL (<205 μmol/L)
3–5 days: <16 mg/dL (<274 μmol/L)
>5 days: <2 mg/dL (<34 μmol/L)

Total bilirubin, full-term neonates—normal range:
0–1 day: <8 mg/dL (<137 μmol/L)
1–2 days: <11.5 mg/dL (<197 μmol/L)
3–5 days: <12 mg/dL (<205 μmol/L)
>5 days: <1.2 mg/dL (<21 μmol/L)

Total bilirubin, adults—normal range:
adults: 0.3–1.3 mg/dL (5.1–22 μmol/L)

Conjugated bilirubin—normal range:
neonates: <0.6 mg/dL (<10 μmol/L)
infants and children: <0.2 mg/dL (<3.4 μmol/L)
adults: 0.1–0.4 mg/dL (1.7–6.8 μmol/L)

To better understand the age-related differences in serum bilirubin concentrations, a brief review of bilirubin metabolism is needed. Bilirubin is a breakdown product of hemoglobin. Hemoglobin, which is released from senescent or hemolyzed RBCs, is degraded by heme oxygenase into iron, carbon monoxide, and biliverdin. Biliverdin undergoes reduction by biliverdin reductase to bilirubin. Unconjugated bilirubin then enters the liver and is conjugated with glucuronic acid to form conjugated bilirubin, which is water soluble. Conjugated bilirubin is excreted in the bile and enters the intestines, where it is broken down by bacterial flora to urobilinogen. However, conjugated bilirubin also can be deconjugated by bacteria in the intestines and reabsorbed back into the circulation.[51]

Compared to adults, newborns have higher concentrations of bilirubin. This results from a higher production of bilirubin in the neonate and a decreased ability to excrete it. A higher rate of production of bilirubin occurs in newborns due to the shorter life span of neonatal RBCs and the higher initial neonatal hematocrit. The average RBC life span is only 65 days in very premature neonates and 90 days in full-term neonates, compared to 120 days in adults.[52] In addition, full-term neonates have a mean hematocrit of about 50%, compared to adult values of approximately 44%. The shorter RBC life span plus the higher hematocrit both increase the load of unconjugated bilirubin to the liver. Newborn infants, however, have a decreased ability to eliminate bilirubin. The activity of neonatal uridine diphosphate glucuronosyltransferase, the enzyme responsible for conjugating bilirubin in the liver, is decreased. In addition, newborns lack the intestinal bacteria needed to breakdown conjugated bilirubin into urobilinogen. However, the newborn's intestine does contain glucuronidase, which can deconjugate bilirubin and allow unconjugated bilirubin to be reabsorbed back into the circulation (enterohepatic circulation). This enterohepatically reabsorbed bilirubin further increases the unconjugated bilirubin load to the liver.

Because of these limitations in bilirubin metabolism, a "physiologic jaundice" commonly occurs in newborns. Typically in full-term neonates, high serum bilirubin concentrations occur in the first few days of life, with a decrease over the next several weeks to values seen in adults. High bilirubin concentrations may occur later in premature newborns, up to the first week of life, and are usually higher and persist longer than in full-term newborns.

Pathologic jaundice may occur in newborns due to many reasons, including increased production of bilirubin, decreased uptake of unconjugated bilirubin into the liver, decreased conjugation of bilirubin in the liver, and increased enterohepatic circulation of bilirubin.[51] Increased production of bilirubin may occur with RBC hemolysis due to blood group incompatibilities, enzyme deficiencies of the erythrocytes, erythrocyte structural defects (e.g., spherocytosis), or in infants of certain racial or ethnic groups (e.g., Asian, Native American, and Greek islander). Certain genetic disorders may cause neonatal hyperbilirubinemia. For example, patients with Gilbert syndrome have decreased hepatic uptake of bilirubin and infants with Crigler-Najjar syndrome have a deficiency of uridine diphosphate glucuronosyltransferase, the enzyme that is responsible for conjugation of bilirubin in the liver. Breastfeeding also is associated with neonatal hyperbilirubinemia and jaundice. Newborns who are exclusively breastfed, not feeding well, or not being enterally fed (i.e., newborns who are not taking anything by mouth) may have increased intestinal reabsorption of bilirubin that can cause or worsen hyperbilirubinemia. Breastfeeding may also increase bilirubin concentrations by other mechanisms. Breast milk may contain substances that decrease the conjugation of bilirubin by inhibiting the enzyme, uridine diphosphate glucuronosyltransferase.

Appropriate monitoring of serum bilirubin is very important in neonates, as high concentrations of unconjugated bilirubin can cause bilirubin encephalopathy or kernicterus (i.e., deposits of bilirubin in the brain). The neurotoxic effects of bilirubin are serious and potentially lethal. Clinical features of acute kernicterus include poor sucking, stupor, seizures, fever, hypotonia, hypertonia, opisthotonus, and retrocollis. Neonates who survive may develop mental retardation, delayed motor skills, movement disorders, and sensorineural hearing loss. Phototherapy and exchange transfusion are common treatments for neonatal hyperbilirubinemia.[51]

AGE-RELATED DIFFERENCES IN HEMATOLOGIC TESTS

Erythrocytes

Mean values and lower limit of normal (minus 2 standard deviations)[53]

Red Blood Cell Count

birth (cord blood): 4.7 (3.9) × 10^{12} cells/L (4.7 (3.9) × 10^{6} cells/μL)
1–3 days: 5.3 (4) × 10^{12} cells/L (5.3 (4) × 10^{6} cells/μL)
1 wk: 5.1 (3.9) × 10^{12} cells/L (5.1 (3.9) × 10^{6} cells/μL)
2 wk: 4.9 (3.6) × 10^{12} cells/L (4.9 (3.6) × 10^{6} cells/μL)
1 mo: 4.2 (3) × 10^{12} cells/L (4.2 (3) × 10^{6} cells/μL)
2 mo: 3.8 (2.7) × 10^{12} cells/L (3.8 (2.7) × 10^{6} cells/μL)
3–6 mo: 3.8 (3.1) × 10^{12} cells/L (3.8 (3.1) × 10^{6} cells/μL)
0.5–2 yr: 4.5 (3.7) × 10^{12} cells/L (4.5 (3.7) × 10^{6} cells/μL)
2–6 yr: 4.6 (3.9) × 10^{12} cells/L (4.6 (3.9) × 10^{6} cells/μL)
6–12 yr: 4.6 (4) × 10^{12} cells/L (4.6 (4) × 10^{6} cells/μL)
12–18 yr, female: 4.6 (4.1) × 10^{12} cells/L (4.6 (4.1) × 10^{6} cells/μL)
12–18 yr, male: 4.9 (4.5) × 10^{12} cells/L (4.9 (4.5) × 10^{6} cells/μL)
18–49 yr, female: 4.6 (4) × 10^{12} cells/L (4.6 (4) × 10^{6} cells/μL)
18–49 yr, male: 5.2 (4.5) × 10^{12} cells/L (5.2 (4.5) × 10^{6} cells/μL)

Hemoglobin

birth (cord blood): 16.5 (13.5) g/dL (10.2 (8.4) mmol/L)
1–3 days: 18.5 (14.5) g/dL (11.5 (9) mmol/L)
1 wk: 17.5 (13.5) g/dL (10.9 (8.4) mmol/L)
2 wk: 16.5 (12.5) g/dL (10.2 (7.8) mmol/L)
1 mo: 14 (10) g/dL (8.7 (6.2) mmol/L)
2 mo: 11.5 (9) g/dL (7.1 (5.6) mmol/L)
3–6 mo: 11.5 (9.5) g/dL (7.1 (5.9) mmol/L)
0.5–2 yr: 12 (10.5) g/dL (7.4 (6.5) mmol/L)
2–6 yr: 12.5 (11.5) g/dL (7.8 (7.1) mmol/L)
6–12 yr: 13.5 (11.5) g/dL (8.4 (7.1) mmol/L)
12–18 yr, female: 14 (12) g/dL (8.7 (7.4) mmol/L)
12–18 yr, male: 14.5 (13) g/dL (9 (8.1) mmol/L)
18–49 yr, female: 14 (12) g/dL (8.7 (7.4) mmol/L)
18–49 yr, male: 15.5 (13.5) g/dL (9.6 (8.4) mmol/L)

Hematocrit

Birth (cord blood): 51 (42)% (0.51 (0.42))
1–3 days: 56 (45)% (0.56 (0.45))
1 wk: 54 (42)% (0.54 (0.42))
2 wk: 51 (39)% (0.51 (0.39))
1 mo: 43 (31)% (0.43 (0.31))
2 mo: 35 (28)% (0.35 (0.28))
3–6 mo: 35 (29)% (0.35 (0.29))
0.5–2 yr: 36 (33)% (0.36 (0.33))
2–6 yr: 37 (34)% (0.37 (0.34))
6–12 yr: 40 (35)% (0.40 (0.35))
12–18 yr, female: 41 (36)% (0.41 (0.36))
12–18 yr, male: 43 (37)% (0.43 (0.37))
18–49 yr, female: 41 (36)% (0.41 (0.36))
18–49 yr, male: 47 (41)% (0.47 (0.41))

Compared to adults, normal newborn infants have higher hemoglobin and hematocrit values. For example, the mean hemoglobin value in a full-term newborn on the first day of life is 18.5 g/dL, compared to 15.5 g/dL in adult males. Hemoglobin and hematocrit start to decrease within the first week of life and reach a minimum level at 8–12 weeks in term infants and by 6 weeks of age in premature infants.[54] This normal decrease in hemoglobin and hematocrit is called the *physiologic anemia of infancy*. This physiologic anemia is normochromic and microcytic and is accompanied by a low reticulocyte count. Physiologic anemia of infancy does not require medical treatment.

The age-related changes in hemoglobin that occur during the first few months of life are due to several reasons. In utero, a low arterial pO_2 exists, which stimulates the production of erythropoietin in the fetus. This results in a high rate of erythropoiesis and accounts for the high levels of hemoglobin and hematocrit that exist at birth. At birth, pO_2 and oxygen content of blood significantly increase with the newborn's first breaths. The higher amount of oxygen that is available to the tissues will down-regulate erythropoietin production and decrease the rate of erythropoiesis.[55] Without the stimulation of erythropoietin to produce new RBCs, hemoglobin concentrations decrease as aged RBCs are removed from the circulation. The shorter life span of neonatal RBCs (90 days versus 120 days in adults) also contributes to the decline in hemoglobin.

Hemoglobin continues to decline in full-term infants until 8–12 weeks of age when values reach 9–11 g/dL. These levels of hemoglobin result in lower amounts of oxygen delivery to the tissues. Usually at this point, oxygen requirements exceed oxygen delivery and the relative hypoxia stimulates the production of erythropoietin. Erythropoiesis then increases and the reticulocyte count and hemoglobin concentrations begin to rise. It is important to remember that the iron from the aged RBCs that were previously removed from the circulation has been stored. The amount of this stored iron is usually adequate to meet the requirements of hemoglobin synthesis.

In premature infants, physiologic anemia occurs at three to six weeks of age (sooner than in full-term infants) and the nadir of the hemoglobin concentrations is lower (e.g., 7–9 g/dL).[55] This can be explained by the even shorter life span of the premature infant's RBCs (40–60 days versus 90 days in full-term newborns) and by the inadequate synthesis of erythropoietin in response to anemia. Thus, anemia of prematurity may require treatment with recombinant human erythropoietin and iron.

Differences in RBC indices also exist for different pediatric ages. For example, compared to adults, newborns have larger erythrocytes (mean corpuscular volume of 108 fL compared to an adult value of 90 fL). Mean values and the lower limits of normal for RBC indices according to different ages are listed in **Table 21-4**.

TABLE 21-4. Red Blood Cell Indices by Age: Mean Values and Lower Limits of Normal (minus 2 standard deviations)

	MCV fL	MCH pg/cell	MCHC g/dL
Birth (cord blood)	108 (98)	34 (31)	33 (30)
1–3 days	108 (95)	34 (31)	33 (29)
1 wk	107 (88)	34 (28)	33 (28)
2 wk	105 (86)	34 (28)	33 (28)
1 mo	104 (85)	34 (28)	33 (29)
2 mo	96 (77)	30 (26)	33 (29)
3–6 mo	91 (74)	30 (25)	33 (30)
0.5–2 yr	78 (70)	27 (23)	33 (30)
2–6 yr	81 (75)	27 (24)	34 (31)
6–12 yr	86 (77)	29 (25)	34 (31)
12–18 yr			
Female	90 (78)	30 (25)	34 (31)
Male	88 (78)	30 (25)	34 (31)
18–49 yr	90 (80)	30 (26)	34 (31)

MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration.
Source: Adapted from reference 53.

Causes of the various types of anemias in pediatric patients are similar to the causes in adults. Of particular note is the iron deficiency anemia that occurs in infants who are fed whole cow's milk. The iron in whole cow's milk is less bioavailable and may cause inadequate iron intake. Typically infants with iron deficiency anemia from whole cow's milk have chronically consumed large amounts of cow's milk (>24 ounces per day) and foods that are not supplemented with iron. Some infants receiving whole cow's milk develop severe iron deficiency due to chronic intestinal blood loss. The blood loss is thought to be due to intestinal exposure to a specific heat-labile protein found in whole cow's milk. Breast feeding, delaying the introduction of whole cow's milk until 12 months of age, and decreasing the amount of whole cow's milk to <24 ounces per day, have been recommended to decrease the loss of blood.[56]

In pediatric patients with anemia, genetic disorders that produce inadequate RBC production (e.g., Diamond-Blackfan anemia), hemolytic anemias (e.g., hereditary spherocytosis), or hemoglobin disorders (e.g., sickle cell disease) also must be considered.

Leukocytes

White Blood Cell Count

Normal range[57]:
neonates 1 day: 9–30 × 10^3 cells/mm^3 (9–30 × 10^9/L)
neonates 2 wk: 5–21 × 10^3 cells/mm^3 (5–21 × 10^9/L)
infants 3 mo: 6–18 × 10^3 cells/mm^3 (6–18 × 10^9/L)
children 0.5–6 yr: 6–15 × 10^3 cells/mm^3 (6–15 × 10^9/L)
children 7–12 yr: 4.5–13.5 × 10^3 cells/mm^3 (4.5–13.5 × 10^9/L)
adults: 4.4–11 × 10^3 cells/mm^3 (4.4–11 × 10^9/L)

Normal WBC counts are higher in neonates and infants compared to adults. Typically in adults, an elevated WBC may indicate infection. However, in neonates and infants with a systemic bacterial infection, the WBC count may be increased, decreased, or within the normal range. Neonates have a lower storage pool of neutrophils and an overwhelming infection (for example, neonatal sepsis) may deplete this pool and cause neutropenia. Therefore, although an increase in WBCs is a

TABLE 21-5. White Blood Cell Differential by Age

	MEAN VALUES			
	NEUTROPHILS	**LYMPHOCYTES**	**EOSINOPHILS**	**MONOCYTES**
Birth	61%	31%	2%	6%
2 wk	40%	63%	3%	9%
3 mo	30%	48%	2%	5%
0.5–6 yr	45%	48%	2%	5%
7–12 yr	55%	38%	2%	5%
Adult	55%	35%	3%	7%

Source: Adapted from reference 57.

nonspecific finding in neonates (i.e., it may occur in many conditions other than sepsis), neutropenia is a highly significant finding and may be the first abnormal laboratory result that indicates neonatal bacterial infection.[54] Not recognizing that neutropenia in neonates indicates a serious infection could result in a delay in treatment and significant morbidity or even mortality for the patient.

In addition to the age-related differences in total WBC count, the age-related differences in WBC differential also need to be taken into consideration when interpreting laboratory results (**Table 21-5**). After the newborn period and up until five to six years of age, lymphocytes represent the most prevalent circulating WBC type. Subsequent to this, neutrophils predominate in the blood for the remainder of life.

Platelets

Platelet Count

Normal range[12]*:*
newborns: 84,000–478,000/mm^3 (84–478 × 10^9/L)
neonate >1 wk, infants, children, adolescents, and adults: 150,000–400,000/mm^3 (150–400 × 10^9/L)

Compared to adults, the normal platelet count in the newborn may be lower. Adult values are reached after one week of age, although platelet counts may range higher in children (up to 600,000/mm^3) than in adults. Platelet counts are discussed in detail in the Chapter 16.

SUMMARY

Interpreting pediatric laboratory data can be complex. Age-related differences in normal reference ranges occur for many common laboratory tests. These differences may be due to changes in body composition and the normal anatomic and physiologic maturation that occurs throughout childhood. Changes in various body compartments, the immature function of the neonatal kidney, and the increased electrolyte and mineral requirements necessary for proper growth, help to explain many age-related differences in serum electrolytes and minerals. Alterations in skeletal muscle mass and the pattern of kidney function maturation account for the various age-related differences in SCr and kidney function tests. Neonatal hepatic immaturity and subsequent maturation help to explain the age-related differences in serum albumin, liver enzymes, and bilirubin. Likewise, the immature hematopoietic system of the newborn and its maturation account for the age-related differences in various hematologic tests.

This chapter also reviews several general pediatric considerations including differences in physiologic parameters, pediatric blood sampling considerations, and the determination of pediatric reference ranges. Interpretation of pediatric laboratory data must take into account the various age-related differences in normal values. If these differences are not taken into consideration, inappropriate diagnoses and treatment may result.

LEARNING POINTS

1. ***Would the dose of a medication that is primarily excreted by the kidney ever have to be adjusted in a patient with a SCr of 0.8 mg/dL?***

   ANSWER: The age of the patient must be taken into consideration when interpreting laboratory tests, especially SCr. In addition, the methodology used to assay SCr needs to be considered. For the Jaffe method, a SCr of 0.8 mg/dL indicates significant renal dysfunction in an infant whose normal SCr should be 0.2–0.4 mg/dL and mild or moderate renal dysfunction in a child whose normal SCr should be 0.3–0.7 mg/dL. However, a SCr of 0.8 mg/dL in an adolescent or adult would be considered within the normal range. Thus, medications that are primarily excreted by the kidney would need to have a dosage adjustment in infants and children with a SCr of 0.8 mg/dL as measured by the Jaffe method. For the IDMS-traceable enzymatic method, measured SCr values and normal values for age will be lower than with the Jaffe method. A SCr of 0.8 mg/dL indicates significant renal dysfunction in an infant or young child (newborn to 4 years of age) whose normal SCr should be 0.03–0.5 mg/dL, and mild-to-moderate renal dysfunction in a child 4–7 years of age whose normal SCr should be 0.03–0.59 mg/dL or in a child 7–10 years of age whose normal SCr should be 0.22–0.59 mg/dL. However, a SCr of 0.8 mg/dL in an adolescent or adult would be considered within the normal range.

2. ***Are there any concerns with using ceftriaxone in a neonate?***

   ANSWER: Ceftriaxone has been shown in vitro to displace bilirubin from its albumin binding sites. Thus, ceftriaxone should not be used in hyperbilirubinemic neonates, especially premature neonates, because displacement of bilirubin from albumin-binding sites may lead to bilirubin encephalopathy. These concerns are so significant that current labeled contraindications to the use of ceftriaxone include premature neonates <41 weeks postmenstrual age and hyperbilirubinemic neonates.[58] In addition, if ceftriaxone is used in a neonate, it should be administered slowly, over 60 minutes, to reduce the risk of bilirubin encephalopathy. Ceftriaxone may also cause sludging in the gallbladder and cholelithiasis. Fatal reactions have been recently reported in neonates due to ceftriaxone–calcium precipitates in the lungs and kidneys when ceftriaxone and calcium-containing IV solutions were coadministered. In some of these cases, the ceftriaxone and calcium-containing solutions were administered in different infusion lines and at different times. Therefore, ceftriaxone must not be administered to neonates who also are receiving (or who are expected to receive) calcium-containing IV solutions, including continuous infusions of parenteral nutrition that contain calcium.[1,58] This is also a labeled contraindication.

3. ***Would a hemoglobin of 9.5 g/dL in a 10-week-old infant who was born at full term require initiation of iron therapy?***

   ANSWER: No. Iron therapy would not be required because this anemia would be considered a normal physiologic anemia of infancy. In full-term infants, hemoglobin values of 9–11 g/dL normally occur at 8–12 weeks of age. After this time, the reticulocyte count and hemoglobin concentration should begin to rise. If an infant's hemoglobin concentration remained at 9.5 g/dL after 12 weeks of age, a further workup of the infant's anemia would be required. If the anemia was found to be due to an iron deficiency, then iron therapy would be required. Dietary causes of iron deficiency, such as consuming large amounts of whole cow's milk, also would need to be considered.

4. ***Differences in TBW, ECW, and ICW occur in neonates (compared to older pediatric patients and adults) and are described in the beginning of this chapter. Could the differences in TBW, ECW, and ICW that occur in neonates have an impact on drug distribution?***

   ANSWER: Yes. Both TBW and ECW, when expressed as a percentage of body weight, are increased in the newborn (especially the premature neonate). The increase in TBW and ECW help explain why water soluble drugs have an increased volume of distribution in these patients. In fact, the volume of distribution for gentamicin, a water soluble drug that primarily distributes to the ECW compartment, correlates well with the volume of ECW. In the neonate, ECW is about 44% of body weight and the volume of distribution for gentamicin is approximately 0.45 L/kg.[1,19,20] Thus, the total amount of body water at birth, distribution between ECW and ICW, and the age-related changes that occur with time, not only impact electrolyte composition and their respective normal laboratory values, but also affect the distribution of medications and required doses.

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22

WOMEN'S HEALTH

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OBJECTIVES

After completing this chapter, the reader should be able to

- Describe how the hypothalamic-pituitary-ovarian axis influences the normal reproductive cycle
- Explain how follicle-stimulating hormone, luteinizing hormone, estradiol, and progesterone affect the menstrual cycle and how they differ in premenopausal women, postmenopausal women, and women after oophorectomy
- Describe clinical symptoms, physical findings, and accompanying laboratory abnormalities in women with secondary amenorrhea
- Describe proposed diagnostic criteria, physical and radiological findings, and accompanying laboratory abnormalities in women with polycystic ovary syndrome
- Describe signs of virilization, causes of hirsutism, and associated laboratory abnormalities
- Describe pertinent medical history, physical examination findings, and laboratory and gynecologic procedures to determine causes of infertility in women
- List and describe how drugs interfere with laboratory values of follicle-stimulating hormone, luteinizing hormone, progesterone, prolactin, and testosterone

ANATOMY AND PHYSIOLOGY

The reproductive cycle depends on the complex cyclic interactions between hypothalamic gonadotropin-releasing hormone (GnRH), the pituitary gonadotropins follicle-stimulating hormone (FSH) and luteinizing hormone (LH), and the ovarian sex steroid hormones estradiol (E_2) and progesterone.[1] Through positive and negative feedback loops, these hormones stimulate ovulation, facilitate implantation of the fertilized ovum, or induce menstruation. Feedback loops between the hypothalamus, pituitary gland, and ovaries are depicted in **Figure 22-1.** If the levels or relationship of any one (or more) of the above hormones become altered, the reproductive cycle becomes disrupted, and ovulation and menstruation cease.

MENSTRUAL CYCLE

The reproductive cycle is divided into three phases: menstruation and the follicular phase, ovulation, and the luteal phase.[1,2] These three phases referring to the status of the ovary during the reproductive cycle are depicted in **Figure 22-2**. The endometrium has the proliferative and secretory phases.

Phase I. Menstruation and the follicular phase. The first day of menstrual bleeding is considered day 1 of the typical 28-day menstrual cycle. During menstruation, the endometrium is sloughed in response to progesterone withdrawal. Women usually menstruate for three to five days. This is accompanied by the development of a new follicle during the follicular phase, with renewal of the endometrial lining of the uterus in preparation for implantation of an embryo.

Menstruation marks the beginning of the follicular phase of the cycle. With the beginning of menstruation, plasma concentrations of E_2, progesterone, and LH reach their lowest point. An increase in FSH begins approximately two days before the onset of menstruation and continues in response to the reduction in negative feedback at the pituitary gland. Under the influence of FSH, the granulosa cells in the ovarian follicle begin to secrete E_2.

E_2 begins to rise in plasma by the fourth day of the cycle. E_2 stimulates LH receptors on the theca cells in the ovarian follicle, further increasing secretion of androgen precursors, which are converted by aromatase to E_2 in granulosa cells. The upregulation of LH receptors and hormone production prepares the granulosa and theca cells for progesterone synthesis after ovulation.

With rising E_2 levels, there is negative feedback to the pituitary gland to decrease the release of FSH and positive feedback to the pituitary gland to increase the release of LH. During the early follicular phase of the cycle, the FSH:LH ratio is <1; as the cycle progresses, the FSH:LH ratio becomes >1, demonstrating both positive and negative feedback effects of E_2 on the pituitary gland.

Phase II. Ovulation. As the dominant follicle secretes more and more E_2, there is marked positive feedback to the pituitary gland to secrete LH. By days 11 to 13 of the normal cycle, an LH surge occurs, which triggers ovulation. Ovulation occurs within 24–36 hours of the LH surge, causing the oocyte to be expelled from the follicle and the follicle to be converted into corpus luteum to facilitate progesterone production

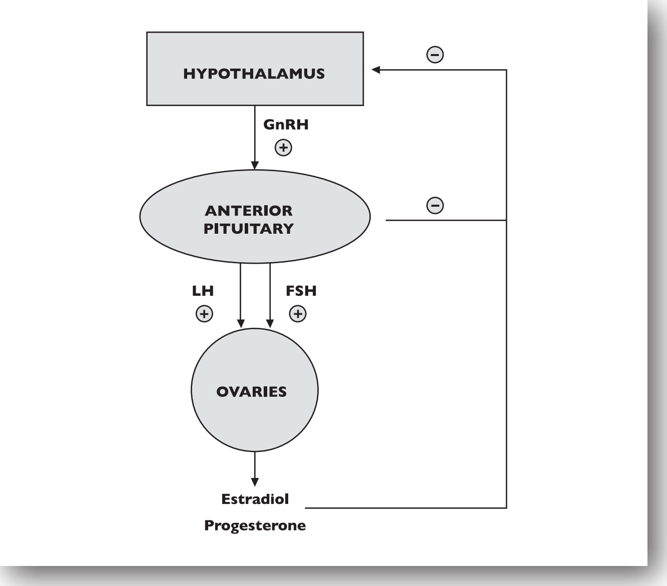

FIGURE 22-1. Hypothalamic-pituitary-ovarian axis (GnRH = gonadotropin-releasing hormone; LH = luteinizing hormone; FSH = follicle-stimulating hormone; (+) = stimulation of hormone secretion; (-) = inhibition of hormone secretion).

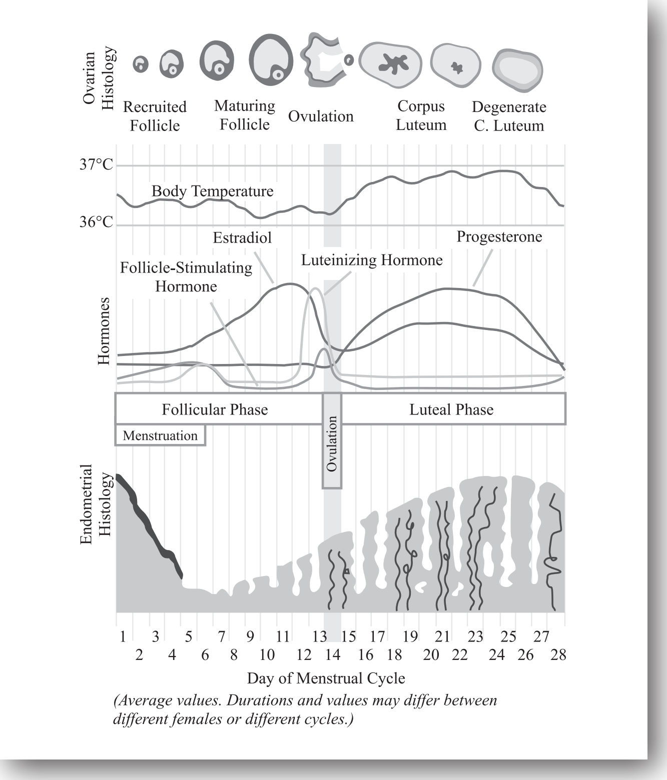

FIGURE 22-2. The menstrual cycle.

during the remainder of the cycle. In addition, there is a slight increase in the basal body temperature (BBT) after ovulation.

Phase III. Luteal phase. The luteal phase of the menstrual cycle is characterized by a change in secretion of sex steroid hormones from E_2 predominance to progesterone predominance. As FSH rises early in the cycle, stimulating production of E_2, additional LH receptors are created in the granulosa cells and then theca cells. With the LH surge at the time of ovulation, LH facilitates production of progesterone.

The production of progesterone begins approximately 24 hours before ovulation and rises rapidly thereafter. A maximum production of progesterone occurs 3–4 days after ovulation and is maintained for approximately 11 days following ovulation. If fertilization and implantation do not occur, progesterone production diminishes rapidly, initiating events leading to the beginning of a new cycle.

Adequate progesterone production is necessary to facilitate implantation of the fertilized oocyte into the endometrium and to sustain pregnancy into the early first trimester. If the initial rise in FSH is inadequate and the LH surge does not achieve maximal amplitude, an “inadequate luteal phase” can occur, resulting in progesterone production that is inadequate to facilitate implantation of a fertilized oocyte or to sustain pregnancy. The corpus luteum has a fixed life span of 13–14 days unless pregnancy occurs. If the oocyte becomes fertilized and implants within the endometrium, the early pregnancy begins secreting human chorionic gonadotropin, which sustains the corpus luteum for another six to seven weeks.

Physiologic plasma levels of progesterone exert negative feedback on pituitary secretion of both FSH and LH. During the luteal phase of the cycle, both FSH and LH are suppressed to low levels. As the corpus luteum fails and progesterone secretion diminishes, FSH begins to rise to prepare a woman for the next reproductive cycle.

AMENORRHEA

Amenorrhea is the absence or abnormal cessation of the menses.[3] Primary and secondary amenorrhea describe the occurrence of amenorrhea before and after menarche, respectively. Primary amenorrhea can be diagnosed if a patient has normal secondary sexual characteristics but no menarche by 16 years of age.[4] Secondary amenorrhea is the absence of menses for three months in women with previously normal menstruation and for nine months in women with previous oligomenorrhea (scant menses). Secondary amenorrhea is more common than primary amenorrhea.[5] The reader is referred to other texts for the evaluation of primary amenorrhea.

The prevalence of amenorrhea not due to pregnancy, lactation, or menopause is approximately 3–4%.[3,5] History, physical examination, and measurement of FSH, thyroid-stimulating hormone (TSH), and prolactin will identify the most common causes of amenorrhea. **Table 22-1** illustrates how symptoms elicited from a patient history assist in diagnosing the cause of amenorrhea.[4,6]

During the physical examination, the clinician should note the presence of galactorrhea, thyromegaly, or other evidence of hypothyroidism or hyperthyroidism, hirsutism, acne, or signs of virilization.[7] In addition, the patient’s body mass index (BMI) should be calculated. A BMI <20 may indicate hypothalamic ovulatory dysfunction, such as occurs with anorexia

TABLE 22-1. Medical History Associated with Amenorrhea

PATIENT HISTORY	ASSOCIATIONS
Acne, greasy or oily skin, hirsutism, obesity	PCOS
History of chemotherapy	Ovarian failure
History of radiation therapy	Ovarian failure
History of diabetes mellitus, Addison disease, or thyroid disease	Ovarian failure (autoimmune etiology)
Galactorrhea	Hyperprolactinemia
Weight loss, excessive exercise, severe dieting	Functional amenorrhea
Sexual activity	Pregnancy
Cessation of menstruation followed by hot flashes, vaginal dryness, dyspareunia, or mood swings	Menopause
Rapid progression of hirsutism	Adrenal or ovarian androgen-secreting tumor
Bodybuilder	Exogenous androgen use
Medication history	Amenorrhea secondary to medication use (e.g., danazol, medroxyprogesterone [Depo-Provera], LHRH agonists, LHRH antagonists, oral contraceptives)
Constipation, hoarseness, loss of hair, memory impairment, sensation of cold, weakness, weight gain	Hypothyroidism
Headache, neurological symptoms, visual field defect	CNS lesion (hypothalamic or pituitary)
History of PID, endometriosis, or D&C	Asherman syndrome
History of cautery for cervical intraepithelial neoplasia or obstructive cervical malignancy	Cervical stenosis
Debilitating illness	Functional amenorrhea

CNS = central nervous system; D&C = dilation and curettage; LHRH = luteinizing hormone-releasing hormone; PCOS = polycystic ovary syndrome; PID = pelvic inflammatory disease.
Source: See references 4 and 6.

TABLE 22-2. Physical Examination Findings Associated with Amenorrhea

FINDINGS	ASSOCIATIONS
Bradycardia	Hypothyroidism, physical or nutritional stress
Coarse skin, coarseness of hair, dry skin, edema of the eyelids, weight gain	Hypothyroidism
Galactorrhea	Hyperprolactinemia
Bradycardia, cold extremities, dry skin with lanugo hair, hypotension, hypothermia, minimum of body fat, orange discoloration of skin (hypercarotenemia)	Anorexia nervosa
Painless enlargement of parotid glands, ulcers or calluses on skin of dorsum of fingers or hands	Bulimia
Signs of virilization: clitoromegaly, frontal balding, increased muscle bulk, severe hirsutism	Adrenal or ovarian androgen-secreting tumor
Centripetal obesity, hirsutism, hypertension, proximal muscle weakness, striae	Cushing syndrome
Increased BMI, hirsutism, acne	PCOS
Transverse vaginal septum, imperforate hymen	Uterine outflow tract obstruction

BMI = body mass index; PCOS = polycystic ovary syndrome.
Source: See references 4 and 6.

or other eating disorders. The presence of breast development suggests there has been previous estrogen activity. Excessive testosterone secretion is suggested most often by hirsutism and rarely by increased muscle mass or signs of virilization. The combination of amenorrhea and galactorrhea strongly correlates with hyperprolactinemia and not virilization. The history and physical examination should include a thorough assessment of the external and internal genitalia. **Table 22-2** illustrates how physical examination findings assist in diagnosing the cause of amenorrhea.[4,6]

Hypothyroidism. Although other clinical signs of thyroid disease are usually noted before amenorrhea presents, abnormal thyroid hormone levels can affect prolactin levels. Treatment of hypothyroidism should restore menses, but this may take several months.[8] **Table 22-3** provides differential diagnoses of anovulatory disorders and associated serum laboratory findings.[9]

Hyperprolactinemia. A patient with markedly elevated prolactin levels, galactorrhea, headaches, or visual disturbances should receive imaging tests to rule out a pituitary tumor. Adenomas are the most common cause of anterior pituitary dysfunction.[10] A prolactin level >100 ng/mL suggests a prolactinoma, and magnetic resonance imaging should be performed. If tumor is excluded as the cause, medications (e.g., oral contraceptive pills, antipsychotics, antidepressants, antihypertensives, histamine blockers, and opiates) are the next most common cause of hyperprolactinemia. Medications usually increase prolactin levels to >100 ng/mL.[10]

In most amenorrheic women with hyperprolactinemia, prolactin levels do not decline without treatment, and the amenorrhea does not resolve as long as the prolactin levels remain elevated.[10] In the absence of another organic condition, dopamine agonists (e.g., bromocriptine) are the preferred treatment of hyperprolactinemia with or without a pituitary tumor.[3,11,12]

Uterine outflow obstruction. The most common cause of outflow obstruction in secondary amenorrhea is Asherman syndrome (intrauterine scarring usually from curettage or infection).[4] Certain gynecologic procedures can help diagnose Asherman syndrome. Other causes of outflow tract obstruction include cervical stenosis and obstructive fibroids or polyps.

Functional (hypothalamic) amenorrhea. Functional disorders of the hypothalamus or higher centers are the most

TABLE 22-3. Differential Diagnosis of Amenorrhea and Associated Serum Laboratory Findings[a]

CONDITION	FSH	LH	PROLACTIN	TESTOSTERONE
Functional amenorrhea secondary to extreme exertion or rapid weight changes	↔	↔	↔	↔
Premature ovarian failure	↑↑↑	↑↑↑↑	↔	↔
Pituitary adenoma	↓	↓	↑↑	↔
Use of progestational agents	↓	↓	↔	↔
Hypothyroidism	↔	↔	↔/↑	↔
Eating disorders	↓↓	↓↓	↔	↔
PCOS	↔/↓	↑↑	↔/↑	↔/↑↑
CAH	↔	↔	↔	↔/↑

CAH = congenital adrenal hyperplasia; FSH = follicle-stimulating hormone; LH = luteinizing hormone; PCOS = polycystic ovary syndrome.

[a] normal = ↔; mildly reduced = ↓; moderately reduced = ↓↓; significantly reduced = ↓↓↓; mildly elevated =↑; moderately elevated =↑↑; significantly elevated = ↑↑↑

Source: Adapted from reference 9.

MINICASE 1

Amenorrhea Secondary to Intense Exercise and Weight Loss

Tonya W., a 34-year-old woman, sees her doctor for a urine pregnancy test due to no menses for about two months. Tonya W. has no significant past medical history. Further questioning reveals she has lost a total of 28 pounds over the past four months and complains of recent acne. Tonya W.'s vital signs include BP 105/70, HR 55 beats/min, temperature 98.8 °F, and weight 112 lb. Laboratory tests are as follows: urine hCG negative, FSH 1 mIU/mL, LH 3 mIU/mL, prolactin 20 ng/mL, and testosterone 20 ng/dL. She also reports heavy exercise for the past year and a decreased appetite over the past six months.

QUESTION: What is the most likely diagnosis?

DISCUSSION: This patient complains of amenorrhea. Pregnancy is not a cause of amenorrhea because her urine hCG is negative. She has decreased appetite, bradycardia, and reports heavy exercise over an extended period of time—all indicative of a functional disorder. The combination of heavy exercise along with nutritional changes such as decreased appetite may contribute to functional amenorrhea.

Treatment of functional amenorrhea depends on the etiology, and it is important to encourage the patient to decrease the intensity of exercise and increase caloric intake. Women with excessive weight loss should be screened for eating disorders and treated if anorexia nervosa or bulimia nervosa is diagnosed. Menses usually will return after a healthy body weight is achieved.[15] With young athletes, menses may return after a modest increase in caloric intake or a decrease in athletic training. Women with amenorrhea also are susceptible to the development of osteoporosis.[16] In the athletic population, this is significant as these women are at an increased risk for stress fractures.[14] Diagnosis is one of exclusion, and the patient should be monitored for improvement before initiating other therapies.

common reason for chronic anovulation. Psychogenic stress, weight changes, undernutrition, and excessive exercise are frequently associated with functional hypothalamic amenorrhea, but the pathophysiologic mechanisms are unclear. More cases of amenorrhea are associated with weight loss than with anorexia, but amenorrhea with anorexia nervosa is more severe.[11,13] Women involved in competitive sports activities have a threefold higher risk of primary or secondary amenorrhea than others, and the highest prevalence is among long-distance runners.[14] (**Minicase 1**.)

Ovarian failure. Approximately 1–5% of women have premature ovarian failure, a condition where persistent estrogen deficiency and elevated FSH levels occur prior to the age of 40 years, resulting in amenorrhea.[17] Ovarian failure is confirmed by documenting an FSH level persistently in the menopausal range.[3] Iatrogenic causes of premature ovarian failure, such as chemotherapy and radiation therapy for malignancy, have a potential for recovery. Ovarian function may fluctuate, with an increasingly irregular menstrual cycle before permanent ovarian failure. The resulting fluctuations in gonadotropin levels account for the lack of accuracy associated with a single FSH value.[18] Women with ovarian failure should be offered estrogen and progestin treatment to promote and maintain secondary sexual characteristics and reduce the risk of developing osteoporosis.

Menopause represents a type of "physiologic" ovarian failure, which is defined as the cessation of menses for at least 12 months. The climacteric or perimenopause are the periods of waning ovarian function before menopause (i.e., the transition from the reproductive to the nonreproductive

MINICASE 2

Perimenopause

Fiona S., a 51-year-old woman, returns for further testing due to complaints of irregular menses over the past seven months, loss of sexual desire, vaginal dryness, and episodes of warmth and sweating throughout the day. Her past medical history includes breast cancer, for which she underwent chemotherapy and radiation. On examination, her BP is 120/68, her HR is 90 beats/min, and her temperature is 100 °F. The thyroid gland is normal to palpation. Cardiac and lung examinations are unremarkable. Breast examination reveals symmetrical breasts, without masses or discharge. Examination of the external genitalia does not reveal any masses. Laboratory values are obtained on day 3 of menses and are as follows: FSH 34 mIU/mL, LH 39 mIU/mL.

QUESTION: What is the most likely diagnosis?

DISCUSSION: This patient complains of irregular menses, vaginal dryness, and intermittent sensations of warmth and sweating. This constellation of symptoms is consistent with perimenopause. Elevated FSH and LH levels confirm the diagnosis. Due to this woman's age and medical history, it is likely she is in the perimenopausal period. However, even when gonadotropins are in the menopausal range, as in this case, ovulation can still be occurring, albeit irregularly and unpredictably. Thus, it is best to draw FSH and LH levels during the follicular phase because they reach their lowest point during this phase. Hot flushes, which are typical vasomotor changes due to decreasing estrogen levels, are associated with skin temperature elevation and sweating lasting for two to four minutes. The low estrogen concentration also decreases epithelial thickness of the vagina, leading to atrophy and dryness. Although her estradiol level would mostly likely be low, it is not a reliable indicator of menopausal transition because estradiol levels are prone to cyclical fluctuations, as shown in Figure 22-2. Thus, it is not necessary to draw an estradiol level.

Treatment for hot flashes includes hormone therapy with estrogen. Certain antidepressants, such as venlafaxine, can help with vasomotor symptoms as well, and paroxetine (Brisdelle) has been recently approved for the treatment of hot flashes in menopause.[19,20] When the woman still has her uterus, the addition of progestin to estrogen replacement is important for preventing endometrial cancer. Because significant increased risks of breast cancer, heart disease, pulmonary embolism, and stroke are associated with hormone therapy, estrogens are not the best treatment for vasomotor symptoms for this patient.[21,22]

TABLE 22-4. Reference Ranges for Serum Hormones According to Age

CATEGORY	FSH (mIU/mL)	LH (mIU/mL)	ESTRADIOL (ng/dL)	PROGESTERONE (ng/dL)
Children	5–10	5–10	<2	
Adult women				
Follicular phase	5–25	5–25	1.8–2.4	37–57
Midcycle	20–30	40–80	16.6–23.2	Rising
Luteal phase	5–25	5–25	6.3–7.3	332–1198
Menopausal women	40–250	>75	<1.5	10–22

FSH = follicle-stimulating hormone; LH = luteinizing hormone.
Source: See reference 23.

years).[1] The average age for menopause in the United States is between 50 and 52 years of age (median 51.5), with 95% of women experiencing this event between the ages of 44 and 55.[1] (**Minicase 2.**)

During perimenopause, the ovarian follicles diminish in number and become less sensitive to FSH.[1] The process of ovulation becomes increasingly inefficient, less regular, and less predictable than in earlier years. Initially, a woman may notice a shortening of the cycle length. With increasing inefficiency of the reproductive cycle, the follicular phase shortens, but the luteal phase is maintained at normal length. With the passing of time, some cycles become anovulatory. As menopause approaches, the remaining follicles become almost totally resistant to FSH. The process of ovulation ceases entirely, and cyclic hormone production ends with menopause.

Serum FSH levels begin to rise with diminishing ovarian function; the elevation is first detected in the follicular phase. This early follicular phase rise in FSH has been developed into an endocrine test of ovarian functional reserve in which one draws an FSH level on cycle day 3. With a further decrease in ovarian function, the FSH level will be elevated consistently throughout the menstrual cycle in perimenopausal women and in women after oophorectomy. **Table 22-4** depicts serum hormones according to age.[23]

The postmenopausal ovary is not quiescent. Under the stimulation of LH, androgens (i.e., testosterone and androstenedione) are secreted. Testosterone concentrations decline after menopause but remain two times higher in menopausal women with intact ovaries than in those with ovaries removed (oophorectomy). Estrone is the predominant endogenous estrogen in postmenopausal women and is termed *extragonadal estrogen*

TABLE 22-5. Steroid Hormone Serum Concentrations in Premenopausal Women, Postmenopausal Women, and Women After Oophorectomy

HORMONE	PREMENOPAUSAL (NORMAL RANGE)	POSTMENOPAUSAL	POSTOOPHORECTOMY
Testosterone (ng/dL)	32 (20–60)	23	11
Androstenedione (ng/dL)	150 (50–300)	80–90	80–90
Estrone (pg/mL)	30–200	25–30	30
Estradiol (pg/mL)	35–500	10–15	15–20

Source: See reference 9.

TABLE 22-6. Suggested Diagnostic Criteria for PCOS

CLINICAL FEATURES

- Amenorrhea, oligomenorrhea, or dysfunctional uterine bleeding
- Anovulatory infertility
- Centripetal obesity
- Hirsutism, acne

ENDOCRINE ABNORMALITIES ON LABORATORY TESTS

- Elevated androgen (i.e., testosterone) levels
- Elevated LH
- Insulin resistance with hyperinsulinemia
- LH-to-FSH ratio >3
- Decreased FSH

RADIOLOGIC ABNORMALITIES ON ULTRASOUND EXAMINATION

- Increased ovarian stromal density and volume
- Multiple (nine or more) subcortical follicular cysts

EXCLUSION OF OTHER ETIOLOGIES

- CAH
- Cushing syndrome
- Prolactinoma
- Virilizing adrenal or ovarian tumors

CAH = congenital adrenal hyperplasia; FSH = follicle-stimulating hormone; LH = luteinizing hormone; PCOS = polycystic ovary syndrome.
Source: See reference 9.

because the concentration is directly related to body weight and androstenedione is converted to estrone in adipose tissue. **Table 22-5** compares concentrations of androgens and estrogens in premenopausal women, postmenopausal women, and women after oophorectomy.[9]

Polycystic ovary syndrome. PCOS affects approximately 6% of women of reproductive age and is the most frequent cause of anovulatory infertility.[24,25] Clinical signs include those associated with a hyperandrogenic anovulatory state, including hirsutism and acne. Approximately 70% of affected women manifest growth of coarse hair in androgen-dependent body regions (e.g., sideburn area, chin, upper lip, periareolar area, chest, lower abdominal midline and thigh) as well as upper-body obesity with a waist-to-hip ratio of >0.85.[9] Patients usually retain normal secondary sexual characteristics and rarely exhibit virilizing signs such as clitoromegaly, deepening of the voice, temporal balding, or masculinization of body habitus. Suggested diagnostic criteria for PCOS are provided in **Table 22-6**.[9]

Women with PCOS often develop polycystic ovaries as a function of a prolonged anovulatory state. Follicular cysts are observed on ultrasound in more than 90% of women with PCOS, but they also are present in up to 25% of normal women.[25-28] Although PCOS is primarily a clinical diagnosis, some debate exists about whether the diagnosis should be based on assays of circulating androgens rather than clinical signs and symptoms of hirsutism and acne, as well as glucose abnormality. This is because a substantial number of women with PCOS have no overt clinical signs of androgen excess.[9]

Laboratory abnormalities in PCOS include elevated levels of testosterone and LH or an elevated LH-to-FSH ratio, an increased LH pulse frequency and altered diurnal rhythm of LH secretion. The LH-to-FSH ratio is used to facilitate diagnosis, and many researchers consider an LH-to-FSH ratio of 3:1 or greater diagnostic of the syndrome.[29,30] Although serum testosterone levels are mildly-to-moderately elevated in women with PCOS, testosterone levels also are measured to rule out virilizing tumors. Prolactin levels are usually measured to exclude a possible prolactinoma. Suggested laboratory and radiologic evaluation for PCOS is provided in **Table 22-7**.[9]

Women with PCOS also should be screened for abnormal glucose metabolism because of an association with glucose intolerance. To aid in the possible prevention of cardiovascular disease, lipid abnormalities and blood pressure should be monitored annually.

The primary treatment for PCOS is weight loss through diet and exercise. Modest weight loss can lower androgen levels, improve hirsutism, normalize menses, and decrease insulin resistance.[30] Use of oral contraceptive pills or cyclic progestational agents can help maintain a normal endometrium. The optimal cyclic progestin regimen to prevent endometrial cancer is unknown, but a monthly 10-day to 14-day regimen is recommended.[30] Insulin sensitizing agents such as metformin can reduce insulin resistance and improve ovulatory function.[30,31]

Hyperandrogenism. Significantly elevated testosterone or Dehydroepiandrosterone sulfate (DHEA-S) levels also may indicate an androgen-secreting tumor (ovarian or adrenal). DHEA-S is an androgen that arises almost exclusively from the adrenal gland. Levels of 17 α-hydroxyprogesterone (17-OHP)

TABLE 22-7. Suggested Laboratory and Radiologic Evaluation of PCOS

17-OHP level[a]
DHEA-S level[a]
Dexamethasone suppression test[a]
Fasting glucose level
FSH level
Lipid profile (total, low-density, and high-density lipoproteins)
LH level
Pelvic ultrasound examination[a]
Prolactin level
Testosterone level
hCG level

17-OHP = 17α-hydroxyprogesterone; DHEAS = dehydroepiandrosterone sulfate; FSH = follicle-stimulating hormone; hCG = human chorionic gonadotropin; LH = luteinizing hormone;
PCOS = polycystic ovary syndrome.
[a]Suggested only in selected patients.
Source: See reference 9.

can help diagnose adult-onset congenital adrenal hyperplasia (CAH).[30] Cushing syndrome is rare; therefore, patients should be screened only when characteristic signs and symptoms (e.g., striae, buffalo hump, significant central obesity, easy bruising, hypertension, proximal muscle weakness) are present.[30]

Estrogen status. If TSH and prolactin levels are normal, a progesterone challenge test can help detect endogenous estrogen that is affecting the endometrium. A withdrawal bleed usually occurs two to seven days after the challenge test.[4] A negative progesterone challenge test signifies inadequate estrogenization, and requires further follow-up for other underlying causes.

HIRSUTISM AND VIRILIZATION

Hirsutism is defined as the presence of excess terminal hair in androgen-dependent areas of a woman's body and occurs in up to 8% of women.[32-34] The disorder is a sign of increased androgen action on hair follicles from increased circulating levels of androgens (endogenous or exogenous) or increased sensitivity of hair follicles to normal levels of circulating androgens. Infrequently, hirsutism may signal more serious pathology, and clinical evaluation should differentiate benign causes from tumors or other conditions that require specific treatment. Hair growth varies widely among women and distinguishing normal variations of hair growth from true hirsutism is important.

Although 60–80% of women with hirsutism have increased levels of circulating androgens, degrees of hirsutism correlate poorly with androgen levels.[35] DHEA-S is an uncommon cause of hirsutism. The ovary is the major source of increased levels of testosterone in women who have hirsutism.[32] Nearly all circulating testosterone is bound to sex hormone binding globulin (SHBG) and albumin, with free testosterone being the most biologically active form. When elevated insulin levels are present, SHBG levels decrease, while free testosterone levels increase. (**Minicase 3**.)

When evaluating hirsutism, it is important to remember that it is only one sign of hyperandrogenism. Other abnormalities associated with excessive levels of androgen include acne, alopecia, android obesity, cardiovascular disease, and dyslipidemia, glucose intolerance/insulin resistance.[32] There are a number of causes of hirsutism. **Table 22-8** lists the causes of hirsutism according to diagnosis and the associated laboratory findings.[32]

Medications that may cause hirsutism include anabolic steroids (e.g., oxymetholone), danazol, metoclopramide,

MINICASE 3

Hirsutism Secondary to Adnexal Tumor

Vivian R., a 45-year-old parous woman, has noticed increasing hair growth on her face and abdomen over the past eight months. She denies using steroid medications, weight change, or a family history of hirsutism. Her menses has been monthly with the exception of the past three months. Her past medical and surgical histories are unremarkable. On examination, her thyroid is normal to palpation. She has excess facial hair and male pattern hair on her abdomen. Acne is noted on the face. She also notes increased sweating and some thinning of her hair. Cardiac and pulmonary examinations are normal. Abdominal examination reveals no masses or tenderness. Examination of the external genitalia reveals possible clitoromegaly. Pelvic examination shows a normal uterus and cervix and an 8 cm, right adnexal mass. Her laboratory values obtained on the fourth day of her menstrual cycle are as follows: LH 10 mIU/mL, FSH 6 mIU/mL, total testosterone 72 ng/dL, and prolactin 13 ng/mL.

QUESTION: What is the mostly likely diagnosis?

DISCUSSION: This patient has onset of excess male-pattern hair over the past six months, as well as features of virilism (clitoromegaly). This is evidence of excess androgens, and the rapid onset suggests a tumor. Adrenal or ovarian tumors are possibilities. She has a large adnexal mass, so the diagnosis is straightforward. Such tumors are normally of low malignant potential and are slow growing.[32] Surgical treatment may be warranted. She has irregular menses because of an inhibition of ovulation by androgens. She does not have the presentation of Cushing syndrome, such as hypertension, buffalo hump, abdominal striae, and central obesity. Likewise, she does not take any medications containing anabolic steroids (e.g., oxymetholone). Although PCOS is probably the most common cause of hyperandrogenism, it does not fit her clinical presentation because it usually presents with a gradual onset of hirsutism and irregular menses since menarche. Also, she does not have an LH:FSH ratio >3.

TABLE 22-8. Causes of Hirsutism and Associated Laboratory Findings[a]

DIAGNOSIS	TESTOSTERONE	17-OHP	LH/FSH	PROLACTIN	DHEAS	CORTISOL
CAH	↔/↑	↑	↔	↔	↔/↑	↔/↓
PCOS	↔/↑	↔	↔/↑ LH ↔/↓ FSH	↔/↑	↔/↑	↔
Ovarian tumor	↑	↔	↔	↔	↔	↔
Adrenal tumor	↑	↔	↔	↔	↑	↔/↑
Pharmacologic agents[b]	↔	↔	↔	↔	↔	↔
Idiopathic	↔	↔	↔	↔	↔	↔
Familial	↔	↔	↔	↔	↔	↔

17-OHP = 17 α-hydroxyprogesterone; CAH = congenital adrenal hyperplasia; DHEAS = dehydroepiandrosterone sulfate; FSH = follicle-stimulating hormone; LH = luteinizing hormone; PCOS = polycystic ovary syndrome.

[a] Normal = ↔; decreased = ↓; increased = ↑.

[b] Pharmacologic agents: androgens (e.g., testosterone, danazol), anabolic steroids (e.g., oxymetholone), metoclopramide, methyldopa, phenothiazines (e.g., prochlorperazine), progestins (e.g., medroxyprogesterone).

Source: See reference 32.

methyldopa, phenothiazines, and progestins.[32] Increased androgen effect that results in hirsutism can be familial; idiopathic; or caused by excess androgen secretion by the ovary (e.g., tumors, PCOS), excess secretion of androgens by adrenal glands (e.g., CAH, Cushing syndrome, tumor), or exogenous pharmacologic sources of androgens.

Idiopathic hirsutism is common and often familial.[32] It is a diagnosis of exclusion and thought to be related to disorders in peripheral androgen activity. Onset occurs shortly after puberty with slow progression. Patients with idiopathic hirsutism generally have normal menses and normal levels of testosterone, 17-OHP, and DHEA-S.

As mentioned previously, PCOS is represented by chronic anovulation and hyperandrogenemia. Patients often report menstrual irregularities, infertility, obesity, and symptoms associated with androgen excess, and diagnosis usually is based on clinical rather than laboratory findings. Up to 70% of patients with PCOS have signs of hyperandrogenism.[32]

CAH is a spectrum of inherited disorders of adrenal steroidogenesis, with decreased cortisol production resulting in overproduction of androgenic steroids.[32,36] The serum 17-OHP measurement is a screening test for adult-onset CAH. Common signs in postadolescent women with adult-onset CAH are hirsutism, acne, and menstrual irregularity. As many as 25% of women with adult-onset CAH also exhibit LH hypersecretion. Serum levels of 17-OHP should be drawn at 8:00 a.m. in the morning when concentrations peak. Basal follicular-phase serum 17-OHP levels >5 ng/mL suggest adult-onset CAH caused by 21-hydroxylase deficiency. In contrast, serum 17-OHP levels are normal in women with PCOS.[37]

Hirsutism may result from use of exogenous pharmacologic agents, including danazol, anabolic steroids (e.g., oxymetholone), and testosterone. Oral contraceptives containing levonorgestrel, norethindrone, and norgestrel tend to have stronger androgen effects, while those with ethynodiol diacetate, norgestimate, drospirenone, and desogestrel are less androgenic.[32]

TABLE 22-9. Signs of Virilization

Acne
Clitoromegaly
Deepening of voice
Hirsutism
Increased libido
Increased muscle mass
Infrequent/absent menses
Loss of breast tissue or normal female body contour
Malodorous perspiration
Temporal hair recession/balding

Source: See reference 32.

Androgen-secreting tumors of the ovary or adrenal are usually heralded by virilization (e.g., development of male characteristics in women) and rapid progression of hirsutism and cessation of menses. Androgen-secreting ovarian tumors are more common than adrenal tumors and are associated with a better prognosis.[32] Virilization occurs in >1% of patients with hirsutism. Signs of virilization are shown in **Table 22-9**.[32]

A thorough history and physical examination are essential to evaluate women with hirsutism to determine which patients need additional diagnostic testing. Family history is important, as 50% of women with hirsutism have a positive family history of the disorder.[32] Physical examination should distinguish normal amounts of hair growth from hirsutism. Diagnosis often can be made on clinical assessment alone or by limited laboratory testing. Virilization should be noted and abdominal and pelvic examinations should be performed to exclude any masses.

Identification of serious underlying disorders is the primary purpose of laboratory testing and should be individualized. About 95% of these patients have PCOS or idiopathic hirsutism.[38] History and physical examination can exclude most underlying disorders, and full hormonal investigation is usually warranted only in those patients with rapid progression of hirsutism, abrupt symptom onset, or virilization.

In patients with hirsutism of peripubertal onset and slow progression, regular menses, otherwise normal physical examination, and no virilization, the likelihood of an underlying neoplasm is small. Whether laboratory investigation in these patients is warranted is controversial; however, some experts recommend routine testing to exclude underlying ovarian and adrenal tumors and adult-onset adrenal hyperplasia.[34] For diagnostic purposes, serum levels of testosterone and 17-OHP are usually sufficient.[32]

For patients with irregular menses, anovulation, PCOS, late-onset adrenal hyperplasia, and idiopathic hirsutism, prolactin levels and thyroid function tests are suggested to identify thyroid dysfunction and pituitary tumors. Testing of glucose, testosterone, and 17-OHP levels should be considered, along with careful breast examination to rule out galactorrhea.

Hirsutism outside of the perimenarchal period, rapid progression of hirsutism, or signs of Cushing syndrome or virilization should indicate the possibility of an ovarian or adrenal neoplasm. Diagnostic testing should examine levels of serum testosterone, 17-OHP, and DHEAS. Levels of serum testosterone >200 ng/dL and DHEAS >700 ng/mL are strongly indicative of virilizing tumors.[39] For patients with this degree of hormonal elevation or those whose history suggests a neoplasm, additional diagnostic imaging, including abdominal computed tomography to assess the adrenals, should be performed.

Pharmacologic treatment for hirsutism aims at blocking androgen action at hair follicles or suppression of androgen production. Classes of pharmacologic agents used include oral contraceptives, antiandrogens (e.g., cyproterone), glucocorticoids, GnRH agonists (e.g., leuprolide), antifungal agents (e.g., ketoconazole), topical hair growth retardants (e.g., depilatory agents), and insulin sensitizing agents (e.g., metformin). Other options include spironolactone and drospirenone-containing contraceptives. Response to pharmacologic agents is slow, occurring over many months.

INFERTILITY

Infertility, occurring in 10–15% of couples in the United States, is defined as one year of frequent, unprotected intercourse during which pregnancy has not occurred or six months for those other than 35 years of age.[7,40] Many of these couples present first to their primary care clinician, who may initiate evaluation and treatment. Infertility can be attributed to any abnormality in the female or male reproductive system and is distributed fairly equally among male factors, ovarian dysfunction, and tubal factors. A smaller percentage of cases are attributed to endometriosis, uterine or cervical factors, or other causes. In approximately one fourth of couples, the cause is uncertain, and the etiology is multifactorial for some couples. Laboratory testing may include serum inhibin b and serum antimuellerian hormone collection. Serum antimuellerian hormone has been shown to be associated with fertility.[41] The reader is referred to other texts for the evaluation of male infertility. (**Minicase 4**.)

The medical history should include details of the menstrual cycle to determine whether the cycles are ovulatory or anovulatory. A menstrual cycle length of 22–35 days suggests ovulatory cycles, as does the presence of mittelschmerz and premenstrual symptoms.[44] During review of the woman's substance use history, caffeine intake should be assessed, as high levels have been associated with lower fertility rates.[44] **Table 22-10** describes important elements in obtaining a medical history and performing a physical examination in women with infertility.[44]

BBT charting is a simple and inexpensive means of documenting ovulation.[45] In recent years, BBT charting for

MINICASE 4

Infertility Secondary to the Male Factor

Beatrice W., a 38-year-old woman, presents with a three-year history of infertility. She states that her menses began at age 14 years, and cycles occur at 28-day intervals. A biphasic BBT chart is recorded. She denies any history of smoking, illicit drug use, or history of sexually transmitted infection. A hysterosalpingogram (HSG) shows patent tubes and a normal uterine cavity. Her husband is 37 years old, and they have been actively trying to conceive for the past three years.

QUESTION: What is the most likely etiology?

DISCUSSION: This patient has secondary infertility. In approaching infertility, there are five basic factors to examine: (1) ovulatory, (2) uterine, (3) tubal, (4) male factor, and (5) peritoneal. She has regular monthly menses, which argues strongly for regular ovulation; the biphasic BBT chart is further evidence for regular ovulation. Uterine and tubal factors are normal based on the normal HSG. If she had prior cryotherapy to the cervix, an examiner might consider cervical factor (rare).[42] Similarly, if she has symptoms of endometriosis (e.g., dysmenorrhea, dyspareunia), then the examiner would focus on the peritoneal factor. For this patient, neither of these are likely, and there is no evidence of a past sexually transmitted disease or potential for pelvic inflammatory disease.

In general, an infertility evaluation is initiated after 12 months of unprotected intercourse during which pregnancy has not been achieved.[43] Because there are no hints favoring one factor over the other for this patient, the clinician should consider evaluation of the male factor. Causes of infertility are fairly equal among male and female reproductive abnormalities.[43] A semen analysis would be recommended for her male partner.

TABLE 22-10. Medical History and Physical Examination in Women with Infertility

HISTORY	PHYSICAL EXAMINATION
Coital practices	Breast formation
Medical history (e.g., genetic disorders, endocrine disorders, PID)	Galactorrhea Genitalia (e.g., patency, masses, tenderness, discharge)
Medications (e.g., hormone therapy)	Hyperandrogenism (e.g., hirsutism, acne, clitoromegaly)
Menstrual history	
Sexually transmitted diseases, genital inflammation (e.g., vaginal discharge, dysuria, abdominal pain, fever)	
Previous fertility	
Substance abuse, including caffeine	
Surgical history (e.g., genitourinary surgery)	
Toxin exposure	

PID = pelvic inflammatory disease.
Source: See reference 44.

TABLE 22-11. Laboratory Evaluation in Women with Infertility

DOCUMENT OVULATION
Measurement of midluteal progesterone level
Urinary LH using home prediction kit
BBT charting
DETERMINE ETIOLOGY IF OVULATORY DYSFUNCTION SUSPECTED
Measurement of FSH, prolactin, TSH, 17-OHP, and testosterone (if hyperandrogenism suspected)
ASSESS OVARIAN RESERVE (WOMEN >35 YEARS OLD)
Measurement of FSH and estradiol levels on day 3 of the menstrual cycle
Clomiphene citrate (Clomid) challenge test

17-OHP = 17α-hydroxyprogesterone; BBT = basal body temperature; FSH = follicle-stimulating hormone; LH = luteinizing hormone; TSH = thyroid-stimulating hormone.
Source: See reference 44.

documentation of ovulation has largely been replaced by use of the less cumbersome urinary LH prediction kit. During ovulatory cycles, an LH surge can be detected in the urine 14–48 hours before ovulation.[46] Additionally, a single midluteal progesterone level, measured at the midpoint between ovulation and the start of the next menstrual cycle, can provide further confirmation as well as information about the adequacy of the luteal phase. A level >6 ng/mL implies ovulation and normal corpus luteal production of progesterone.[47] Of the three tests, the urinary LH kit provides the greatest accuracy in predicting ovulation.[47]

If ovulatory dysfunction is suspected based on the results of initial evaluation, focused laboratory investigation and other testing can help determine the underlying cause. Testing in patients with amenorrhea, irregular menses, or galactorrhea should involve checking FSH, prolactin, and TSH levels.[43,48] Low or normal FSH levels are most common in patients with PCOS and hypothalamic amenorrhea.[43] The presence or absence of obesity and androgenization, generally occurring in women with PCOS, can be used to distinguish between the two disorders.[43] A high FSH level suggests possible ovarian failure.[43] Evaluation of a prolactin level is useful to rule out pituitary tumor, and measurement of TSH is necessary to rule out hypothyroidism.[43] Measurement of 17-OHP and serum testosterone levels is helpful in evaluating patients with hyperandrogenism or late-onset CAH and androgen-secreting tumors.[49] **Table 22-11** describes the key laboratory evaluations and specialized tests that should be performed in a woman with infertility.[44]

Women older than 35 years may benefit from testing of FSH and E_2 levels on day 3 of their menstrual cycle to assess ovarian reserve.[50] An FSH level of >10 mIU/mL, combined with E_2 level of >80 pg/mL, suggests favorable follicular potential.[50] The clomiphene citrate challenge test—in which the FSH level is obtained on day 3 of the cycle, then again on day 10 after administration of clomiphene citrate 100 mg/day on days 5 to 9—also can be helpful in assessing ovarian reserve.[50] Normal and abnormal values vary by laboratory.

If the initial history and physical examination suggest tubal dysfunction or a uterine abnormality, or if other testing has failed to reveal an etiology, hysterosalpingography, a radiologic study in which dye is placed into the uterine cavity via a transcervical catheter), is indicated.[43,48] The contour of the uterine cavity, including the presence or absence of any abnormalities, as well as tubal patency can be assessed. Other gynecologic procedures can be performed to further detect tubal or uterine abnormalities.

Management of infertility involves treating the couple, treating the male partner (if infertile) and treating the female (if infertile). Couple management includes reviewing coital frequency, the "fertile window," use of ovulation kits, and emotional support. For men, they should be referred to a fertility specialist for evaluation of possible semen abnormalities. Women should be treated according to the underlying etiology, whether it is ovulatory dysfunction, tubal/uterine/pelvic disease, or unexplained infertility.

In women with anovulation resulting from a specific condition such as thyroid dysfunction, the underlying cause should be corrected if possible.[43] Women with hyperprolactinemia can be treated with dopaminergic agents (e.g., bromocriptine), which may restore ovulation.[51] Insulin-sensitizing agents, such as metformin, have been shown to increase ovulation and pregnancy rates in patients with PCOS.[52] In other women with ovulatory dysfunction without evident cause or that is not otherwise correctable, the condition can be managed with the use of oral ovulation-inducing agents such as clomiphene citrate and aromatase inhibitors such as letrozole.[53] Tubal disease may be treated with tubal reparative surgery or with in vitro fertilization

(IVF).[44,54] Patients with endometriosis may benefit from laparoscopic ablation or ovulation induction with or without IVF.[55]

LABORATORY TESTS

Follicle-Stimulating Hormone[23]

Children = 5–10 mIU/mL (5–10 IU/L)
Adult women, follicular phase = 5–25 mIU/mL (5–25 IU/L)
Adult women, midcycle = 20–30 mIU/mL (20–30 IU/L)
Adult women, luteal phase = 5–25 mIU/mL (5–25 IU/L)
Menopausal women = 40–250 mIU/mL (40–250 IU/L)

Follicle-stimulating hormone (FSH) is a glycoprotein pituitary hormone produced and stored in the anterior pituitary. It is under complex regulation by hypothalamic GnRH and by the gonadal sex hormones estrogen and progesterone. Normally, FSH increases occur at earlier stages of puberty, two to four years before LH reaches comparable levels. In females, FSH stimulates follicular formation in the early stages of the menstrual cycle; then the midcycle surge of LH causes ovulation of the FSH-ripened ovarian follicle.

This test may be helpful in determining whether a gonadal deficiency is of primary origin or is secondary to insufficient stimulation by the pituitary hormones. Decreased FSH levels may occur in feminizing and masculinizing ovarian tumors (when FSH production is inhibited because of increased estrogen secretion); pituitary adenomas; neoplasm of the adrenal glands (which influences secretion of estrogen or androgens); and PCOS.[56] Increased FSH levels occur in premature ovarian failure and in menopause.[56]

The date of the last menstrual period (LMP) should be considered when interpreting FSH in premenopausal women. Sometimes multiple blood specimens are necessary because of episodic releases of FSH from the pituitary gland. An isolated sample may not indicate the actual activity; therefore, multiple single blood specimens may be required.

Interfering factors include recently administered radioisotopes, hemolysis of the blood sample, pregnancy, and drugs, such as estrogens or oral contraceptives or testosterone. **Table 22-12** provides a detailed list of drugs affecting plasma laboratory test values of FSH.[56]

Luteinizing Hormone[23]

Children = 5–10 mIU/mL (5–10 units/L)
Adult women, follicular phase = 5–25 mIU/mL (5–25 units/L)
Adult women, midcycle = 40–80 mIU/mL (40–80 units/L)
Adult women, luteal phase = 5–25 mIU/mL (5–25 units/L)
Menopausal women = >75 mIU/mL (>75 units/L)

Like FSH, *luteinizing hormone* (LH) is a glycoprotein pituitary hormone produced and stored in the anterior pituitary that is under complex regulation by hypothalamic-releasing hormone and by estrogen and progesterone. The midcycle surge of LH causes ovulation. Decreased LH and FSH occur in pituitary adenomas and eating disorders, while elevated levels are found in ovarian failure and menopause.[56] Elevated basal LH with an LH/FSH ratio of 3 or more and some increase of ovarian androgen in an essentially anovulatory adult woman is presumptive evidence of PCOS.[56]

The date of the LMP should be considered in premenopausal females. Interfering factors include recently administered radioisotopes, hemolysis of the blood sample, pregnancy, and estrogens or oral contraceptives or testosterone.[56] A detailed list of drugs that affect plasma laboratory test values of LH are listed in **Table 22-13.**[56]

TABLE 22-12. Drugs Affecting Plasma Laboratory Test Values of FSH

INCREASE FSH (OR CAUSE FALSE-POSITIVE VALUES)	DECREASE FSH (OR CAUSE FALSE-NEGATIVE VALUES)
Phenytoin	Anabolic steroids (e.g., danazol)
Dopamine agonists (bromocriptine, levodopa)	Carbamazepine
Cimetidine	Diethylstilbestrol
GnRH agonists	Digoxin
Growth hormone-releasing hormone antagonists	Estrogen/oral contraceptives
Ketoconazole	Megestrol
Naloxone	Phenothiazines (e.g., promethazine)
Pravastatin	Pravastatin
Spironolactone	Tamoxifen
Tamoxifen	Testosterone

FSH = follicle-stimulating hormone; GnRH = gonadotropin-releasing hormone.
Source: See reference 56.

TABLE 22-13. Drugs Affecting Plasma Laboratory Test Values of LH

INCREASE LH (OR CAUSE FALSE-POSITIVE VALUES)	DECREASE LH (OR CAUSE FALSE-NEGATIVE VALUES)
Anticonvulsants (e.g., phenytoin)	Anabolic steroids (e.g., danazol)
Dopamine agonists (bromocriptine)	Anticonvulsants (e.g., carbamazepine)
Clomiphene	Corticotropin-releasing hormone
GnRH agonists	Diethylstilbestrol
Growth hormone-releasing hormone antagonists	Digoxin
Ketoconazole	Dopamine agonists
Mestranol	Estrogen/oral contraceptives
Spironolactone	Megestrol
	Phenothiazines (e.g., thioridazine)
	Pravastatin
	Progesterone
	Tamoxifen
	Testosterone

GnRH = gonadotropin-releasing hormone; LH = luteinizing hormone.
Source: See reference 56.

Estradiol[23]

Children = <2 ng/dL
Adult women, follicular phase = 1.8–2.4 ng/dL (66–88 pmol/L)
Adult women, midcycle = 16.6–23.2 ng/dL (609–852 pmol/L)
Adult women, luteal phase = 6.3–7.3 ng/dL (231–268 pmol/L)

Together with the FSH levels, *estradiol* (E_2) is useful in evaluating menstrual and fertility problems, as well as estrogen-producing tumors. E_2 is the most active of the endogenous estrogens. *Estriol* (E_3) levels in both plasma and urine rise as pregnancy advances; significant amounts are produced in the third trimester. E_3 is no longer considered useful for detection of fetal distress.[56]

E_2 levels are increased by estrogen-producing tumors, during menstruation, before ovulation and during the 23rd to 41st weeks of pregnancy. E_2 levels are decreased in premature ovarian failure and in menopause. Normal E_2 values vary widely between women and in the presence of pregnancy, the menopausal state, or the follicular, ovulatory, or luteal stage of the menstrual cycle.

The number of weeks of gestation should be considered if the patient is pregnant when interpreting E_2 levels. The number of days into the menstrual cycle must be documented and considered for a nonpregnant woman. Interfering factors include radioactive pharmaceuticals and oral contraceptives.

Progesterone[23]

Adult women, early in cycle = 37–57 ng/dL (1.2–1.8 nmol/L)
Adult women, midcycle = rising
Adult women, luteal phase = 332–1198 ng/dL (10.6–38.1 nmol/L)
Menopausal women = 10–22 ng/dL (0.3–0.7 nmol/L)

Progesterone is primarily involved in the preparation of the uterus for pregnancy and its maintenance during pregnancy. The placenta begins producing progesterone at 12 weeks of gestation. Progesterone level peaks in the midluteal phase of the menstrual cycle. In nonpregnant women, progesterone is produced by the corpus luteum. Progesterone on day 21 is the single best test to determine whether ovulation has occurred.

This test is part of a fertility workup to confirm ovulation, evaluate corpus luteum function, and assess risk for early spontaneous abortion. Testing of several samples during the cycle is necessary. Ovarian production of progesterone is low during the follicular (first) phase of the menstrual cycle. After ovulation, progesterone levels rise for two to five days and then fall. During pregnancy, there is a gradual increase from week 9 to week 32 of gestation, often to 100 times the level in the nonpregnant woman. Levels of progesterone in twin pregnancy are higher than in a single pregnancy.

Increased progesterone levels are associated with CAH and some ovarian tumors. Decreased progesterone levels are associated with threatened spontaneous abortion and hyperprolactinemia.

The date of the LMP and length of gestation should be recorded. No radioisotopes should be administered within one week before the test. Drugs that affect plasma laboratory test values of progesterone are listed in **Table 22-14.**[56]

TABLE 22-14. Drugs Affecting Plasma Laboratory Test Values of Progesterone

INCREASE PROGESTERONE (OR CAUSE FALSE-POSITIVE VALUES)	DECREASE PROGESTERONE (OR CAUSE FALSE-NEGATIVE VALUES)
Valproic acid	Ampicillin
Clomiphene	Anticonvulsants (carbamazepine, phenytoin)
Corticotropin	Danazol
Ketoconazole	GnRH agonists (e.g., leuprolide)
Progesterone	Oral contraceptives
Tamoxifen	Pravastatin

GnRH = gonadotropin-releasing hormone.
Source: See reference 56.

Prolactin[23]

Children = 1–20 ng/mL (1–20 mcg/L)
Adult women = 1–25 ng/mL (1–25 mcg/L)
Menopausal women = 1–20 ng/mL (1–20 mcg/L)

Prolactin is a pituitary hormone essential for initiating and maintaining lactation. The gender difference in prolactin does not occur until puberty when increased estrogen production results in higher prolactin levels in females. A circadian change in prolactin concentration in adults is marked by episodic fluctuation and a sleep-induced peak in the early morning hours.[56]

This test may be helpful in the diagnosis, management, and follow-up of prolactin-secreting tumors, including the effectiveness of surgery, chemotherapy, and radiation treatment. Levels >100 ng/mL in a nonlactating female indicates a prolactin-secreting tumor; however, a normal prolactin level does not rule out pituitary tumor. In addition to pituitary adenomas, increased prolactin levels are associated with hypothyroidism (primary), PCOS, and anorexia nervosa, and these increased levels are helpful in the differential diagnosis of infertility.

The patient should be fasting for 12 hours before testing. Specimens should be procured in the morning, three to four hours after awakening. Interfering factors occur in a number of circumstances. Increased values are associated with newborns, pregnancy, the postpartum period, stress, exercise, sleep, nipple stimulation, and lactation. Drugs, such as estrogens, methyldopa, phenothiazines, and opiates, may increase values. Dopaminergic drugs inhibit prolactin. Administration of dopaminergic agents can normalize prolactin levels in patients with galactorrhea, hyperprolactinemia, and pituitary tumor. **Table 22-15** provides a comprehensive list of drugs affecting plasma laboratory test values of prolactin.[56,57]

Testosterone[23]

Children = 0.12–0.16 ng/mL (0.4–0.6 nmol/L)
Adult women = 0.2–0.6 ng/mL (0.7–2.1 nmol/L)
Menopausal women = 0.21–0.37 ng/mL (0.7–1.3 nmol/L)

TABLE 22-15. Drugs Affecting Plasma Laboratory Test Values of Prolactin

INCREASE PROLACTIN (OR CAUSE FALSE-POSITIVE VALUES)	DECREASE PROLACTIN (OR CAUSE FALSE-NEGATIVE VALUES)
Antihistamines	Calcitonin
Antipsychotics (AP) (e.g., Typical AP haloperidol)	Carbamazepine
Antipsychotics (AP) (e.g., Atypical AP aripiprazole)	Clonidine
Calcitonin	Cyclosporin A
Cimetidine	Dexamethasone
Danazol	Dopamine agonists (apomorphine, bromocriptine, levodopa)
Diethylstilbestrol	Ergot alkaloid derivatives
Estrogens/oral contraceptives	Nifedipine
Fenfluramine	Opiates (morphine)
Furosemide	Pergolide
GnRH agonists	Ranitidine
Growth hormone-releasing hormone antagonists	Rifampin
Histamine antagonists	Secretin
Insulin	Tamoxifen
Interferon	
Labetalol	
Loxapine	
Megestrol	
Methyldopa	
Metoclopramide	
Monoamine oxidase inhibitors	
Molindone	
Nitrous oxide	
Opiates (e.g., morphine)	
Parathyroid hormone	
Pentagastrin	
Phenothiazines (e.g., chlorpromazine)	
Phenytoin	
Ranitidine	
Reserpine	
SNRIs (e.g., venlafaxine)	
SSRIs (e.g., sertraline) Thiothixene	
Thyrotropin-releasing hormone	
Tumor necrosis factor	
Verapamil	

GnRH = gonadotropin-releasing hormone; SRNIs = serotonin-norepinephrine reuptake inhibitors; SSRIs = selective serotonin reuptake inhibitors.
Source: See references 56, 57.

TABLE 22-16. Drugs Affecting Plasma Laboratory Test Values of Testosterone

INCREASE TESTOSTERONE (OR CAUSE FALSE-POSITIVE VALUES)	DECREASE TESTOSTERONE (OR CAUSE FALSE-NEGATIVE VALUES)
Anabolic steroids (e.g., danazol)	Alcohol
Anticonvulsants (e.g., phenytoin, barbiturates, rifampin)	Anticonvulsants (e.g., carbamazepine)
Cimetidine	Androgens
Clomiphene	Cimetidine
Dopamine agonists (e.g., bromocriptine)	Corticosteroids (e.g., dexamethasone)
Gonadotropin	Cyclophosphamide
Pravastatin	Diazoxide
Tamoxifen	Diethylstilbestrol
	Digoxin
	Estrogens/oral contraceptives
	Ketoconazole
	GnRH agonists (e.g., leuprolide)
	Magnesium sulfate
	Medroxyprogesterone
	Phenothiazines (e.g., thioridazine)
	Pravastatin
	Spironolactone
	Tetracycline

GnRH = gonadotropin-releasing hormone.
Source: See reference 56.

The adrenal glands and ovaries in women secrete *testosterone*. Excessive production virilizes women. Testosterone exists in serum as both unbound (free) fractions and fractions bound to albumin, SHBG, and testosterone-binding globulin. Unbound (free) testosterone is the active portion. Testosterone serum levels undergo large and rapid fluctuations; levels peak in early morning.[56]

This test is useful in the detection of ovarian tumors and virilizing conditions in women. It also may be part of a fertility workup. Increased total testosterone levels occur in adrenal neoplasms, CAH, and ovarian tumors (benign or malignant). Increased free testosterone levels are associated with female hirsutism, PCOS, and virilization.

Blood should be drawn in the early morning (between 6:00 a.m. and 10:00 a.m.) to obtain the highest levels. Multiple pooled samples drawn at different times throughout the day may be necessary for more reliable results. No radioisotopes should be administered within one week before the test. A number of drugs interfere with test results, including estrogen, androgens, and steroids, which decrease testosterone levels. Other drugs that interfere with interpreting laboratory values of testosterone are provided in **Table 22-16**.[52,57]

SUMMARY

Knowledge of the HPO axis is key to understanding the normal reproductive cycle throughout a woman's lifespan, including pubertal development, menstruation, pregnancy, and menopause. Changes in the gonadotrophic hormones FSH and LH and the ovarian steroid hormones E_2 and progesterone also are essential in identifying underlying causes of amenorrhea, hirsutism, and infertility. Moreover, pharmacologic treatments are often based on alteration of the HPO axis.

LEARNIING POINTS

1. ***What laboratory abnormalities are useful in the differential diagnosis of secondary amenorrhea?***

   ANSWER: In addition to history and physical examination, FSH, TSH, and prolactin will identify the most common causes of amenorrhea.[3] When the physical examination is normal, the initial investigations should exclude pregnancy. When FSH values are normal or low, the problem is most often PCOS, or functional (hypothalamic) amenorrhea due to anorexia or extreme exercise. Conversely, an elevated FSH would indicate premature ovarian failure or menopause. Measurement of TSH is useful to rule out subclinical hypothyroidism, even in the absence of thyroid-related symptoms. If the serum prolactin is persistently elevated and there is no history of medication or drug use that may elevate prolactin, a pituitary tumor should be considered.

2. ***What laboratory evaluations can be performed to confirm if patient symptoms are related to the perimenopausal period?***

   ANSWER: A history and physical examination are helpful when evaluating women who may be in the perimenopausal period. Women often present with a triad of symptoms including irregular menses, feelings of inadequacy, and sensations of warmth and sweating. In the United States, 95% of women will experience perimenopause between the ages of 44 and 55.[1] FSH and LH levels can be drawn to confirm diagnosis; however, it is important to draw the levels during the follicular phase as they are lowest during this time. E_2 levels are not reliable indicators of menopausal transition as they fluctuate over time.[23]

3. ***What laboratory evaluations can be done to detect women with ovulatory infertility?***

   ANSWER: Ovulation disorders constitute 40% of cases of female infertility.[44] To document ovulation, midluteal progesterone levels can be obtained in addition to urinary LH using home prediction kits and BBT charting. If ovulatory dysfunction is suspected based on results of initial evaluations, focused laboratory investigation can help determine the underlying cause. FSH, prolactin, TSH and testosterone levels can help identify functional (hypothalamic) amenorrhea, pituitary tumor, thyroid disease, and adrenal disease. Low or normal FSH levels are most common in patients with PCOS and hypothalamic amenorrhea, such as occurs with extreme exertion or in eating disorders. Elevated FSH can identify premature ovarian failure. To assess ovarian reserve, measurement of FSH and E_2 levels on day 3 of the menstrual cycle or the clomiphene citrate challenge test may be done.

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QUICKVIEW | FSH

PARAMETER	DESCRIPTION	COMMENTS
Reference range		
Children	5–10 mIU/mL (5–10 IU/L)	Sometimes multiple blood specimens are necessary because of episodic increases of FSH
Adult women, follicular phase	5–25 mIU/mL (5–25 IU/L)	
Adult women, midcycle	20–30 mIU/mL (20–30 IU/L)	Document date of LMP
Adult women, luteal phase	5–25 mIU/mL (5–25 IU/L)	
Menopausal women	40–250 mIU/mL (40–250 IU/L)	
Critical values	Not established	Extremely high or low values should be reported quickly
Natural substance?	Yes	
Inherent action?	Yes	
Major causes of…		
High results		
Associated signs and symptoms	Premature ovarian failure	Hot flashes/night sweats
	Menopause	Hot flashes/night sweats
Low results		
Associated signs and symptoms	Ovarian tumors	Virilization
	Pituitary adenoma	Galactorrhea/visual change
	Adrenal tumors	Virilization
	PCOS	Hirsutism/acne/obesity
	Eating disorders	Cachectic/decreased BMI
After insult, time to…	Not applicable	
Initial evaluation		
Peak values		
Normalization		
Diseases monitored with test	Infertility	FSH >10 mIU/mL on day 3 of the menstrual cycle suggests normal ovarian reserve
Drugs monitored with the test	Clomiphene challenge test	Compares FSH before and after clomiphene administration to determine ovarian reserve
Significant interferences with laboratory tests	Recently administered radioisotopes Hemolysis of blood sample Estrogens, oral contraceptives, testosterone, progestational agents (Table 22-12) Pregnancy	

BMI = body mass index; FSH = follicle-stimulating hormone; LMP = last menstrual period; PCOS = polycystic ovary syndrome.

QUICKVIEW | LH

PARAMETER	DESCRIPTION	COMMENTS
Reference range		
Children	5–10 mIU/mL (5–10 units/L)	Often measured with FSH to determine hormonally related functions/disorders
Adult women, follicular phase	5–25 mIU/mL (5–25 units/L)	
Adult women, midcycle	40–80 mIU/mL (40–80 units/L)	Document date of LMP
Adult women, luteal phase	5–25 mIU/mL (5–25 units/L)	
Menopausal women	>75 mIU/mL (7–75 units/L)	
Critical values	Not established	Extremely high or low values should be reported quickly
Natural substance?	Yes	
Inherent action?	Yes	
Major causes of…		
High results		
Associated signs and symptoms	Premature ovarian failure	Hot flashes/night sweats
	Menopause	Hot flashes/night sweats
	PCOS	Hirsutism/acne/obesity
Low results		
Associated signs and symptoms	Pituitary adenoma	Galactorrhea/visual changes
	Eating disorders	Cachectic/low BMI
After insult, time to…		
Initial evaluation	Not applicable	
Peak values		
Normalization		
Diseases monitored with test	None	
Drugs monitored with the test	None	
Significant interferences with laboratory tests	Recently administered radioisotopes	
	Hemolysis of blood sample	
	Estrogens or oral contraceptives	
	Progestational agents, testosterone (Table 22-13)	
	Pregnancy	

BMI = body mass index; FSH = follicle-stimulating hormone; LH = luteinizing hormone; LMP = last menstrual period.

QUICKVIEW | Estradiol (E_2)

PARAMETER	DESCRIPTION	COMMENTS
Reference range		
Children	<2 ng/dL	Estradiol is most active of endogenous estrogens
Adult women, early cycle	1.8–2.4 ng/dL	
Adult women, midcycle	16.6–23.2 ng/dL	Document date of LMP and length of gestation
Adult women, luteal phase	6.3–7.3 ng/dL	
Critical values	Not established	Extremely high or low values should be reported quickly
Natural substance?	Yes	
Inherent action?	Yes	
Major causes of…		
High results		
Associated signs and symptoms	Estrogen-producing tumors	
	Menstruation/preovulatory	
	23rd to 41st weeks of pregnancy	
Low results		
Associated signs and symptoms	Menopause	Hot flashes/night sweats
	Premature ovarian failure	Hot flashes/night sweats
After insult, time to…	Not applicable	
Initial evaluation		
Peak values		
Normalization		
Diseases monitored with test	Fertility	Estradiol >8 ng/dL on day 3 suggests adequate ovarian reserve
Drugs monitored with the test	None	
Significant interferences with laboratory tests	Radioactive pharmaceuticals and oral contraceptives	

LMP = last menstrual period.

QUICKVIEW | Progesterone

PARAMETER	DESCRIPTION	COMMENTS
Reference range		
Adult women, early cycle	37–57 ng/dL (1.2–1.8 nmol/L)	Document LMP and length of gestation
Adult women, midcycle	Rising	
Adult women, luteal phase	332–1198 ng/dL (10.6–38.1 nmol/L)	
Menopausal women	10–22 ng/dL (0.3–0.7 nmol/L)	
Critical values	Not established	Extremely high or low values should be reported quickly
Natural substance?	Yes	
Inherent action?	Yes	
Major causes of…		
High results		
Associated signs and symptoms	CAH	↑ 17-OHP, ↑DHEAS, ↓cortisol, hirsutism/acne
	Ovarian tumor	Virilization, rapid progression of symptoms
Low results		
Associated signs and symptoms	Spontaneous abortion	Vaginal bleeding
After insult, time to…	Not applicable	
Initial evaluation		
Peak values		
Normalization		
Diseases monitored with test	Infertility	Midluteal progesterone >6 ng/mL suggests ovulation
Drugs monitored with the test	None	
Significant interferences with laboratory tests	Drugs may affect test outcome (Table 21-14)	

17-OHP = 17α-hydroxyprogesterone; CAH = congenital adrenal hyperplasia; DHEAS = dehydroepiandrosterone sulfate; LMP = last menstrual period.

QUICKVIEW | Prolactin

PARAMETER	DESCRIPTION	COMMENTS
Reference range		
Children	1–20 ng/mL (1–20 mcg/L)	Obtain 12-hr fasting samples in the morning
Adult women	1–25 ng/mL (1–25 mcg/L)	
Menopausal women	1–20 ng/mL (1–20 mcg/L)	
Critical values	Levels >100 ng/mL in nonlactating female may indicate a prolactin-secreting tumor	Extremely high or low values should be reported quickly
Natural substance?	Yes	
Inherent action?	Yes	
Major causes of…		
High results		
Associated signs and symptoms	Pituitary adenoma	Galactorrhea/visual changes
	Hypothyroidism (primary)	Coarse skin and hair
	PCOS	Hirsutism/acne/obesity
	Anorexia nervosa	Cachectic/low BMI
Low results		
Associated signs and symptoms	No common disorders	
After insult, time to…	Not applicable	
Initial evaluation		
Peak values		
Normalization		
Diseases monitored with test	Pituitary adenoma	
Drugs monitored with the test	Dopaminergic drugs	To monitor effect on prolactin levels in pituitary adenoma
Significant interferences with laboratory tests	Increased values are associated with newborns, pregnancy, postpartum period, stress, exercise, sleep, nipple stimulation, and lactation Drugs (estrogens, methyldopa, phenothiazines, opiates) may increase values (Table 22-15)	

BMI = body mass index; PCOS = polycystic ovary syndrome.

QUICKVIEW | Testosterone

PARAMETER	DESCRIPTION	COMMENTS
Reference range		
Children	0.12–0.16 ng/mL (0.4–0.6 nmol/L)	Unbound (free) testosterone is active form
Adult women	0.2–0.6 ng/dL (0.7–2.1 nmol/L)	Draw levels between 6:00 a.m. and 10:00 a.m.
Menopausal women	0.21–0.37 ng/dL (0.7–1.3 nmol/L)	
Critical values	Testosterone >200 ng/dL indicates virilizing tumor	Extremely high or low values should be reported quickly
Natural substance?	Yes	
Inherent action?	Yes	
Major causes of…		
High results		
Associated signs and symptoms	Adrenal neoplasms	Virilization, ↑DHEAS, ↑cortisol, rapid progression of symptoms
	CAH	↑17-OHP, ↑DHEAS, ↓cortisol, hirsutism
	Ovarian tumors	Virilization, rapid progression of symptoms
	PCOS	Hirsutism/acne/obesity, ↑DHEAS
	Cushing syndrome	Buffalo hump/obesity/striae
Low results		
Associated signs and symptoms	No common disorders	
After insult, time to…	Not applicable	
Initial evaluation	Rapid progression of symptoms indicative of ovarian or adrenal tumor	
Peak values		
Normalization		
Diseases monitored with test	None	
Drugs monitored with the test	None	
Significant interferences with laboratory tests	Estrogen therapy increases testosterone levels (Table 22-16) Many drugs, including androgens and steroids, decrease testosterone levels (Table 22-16)	

17-OHP = 17α-hydroxyprogesterone; CAH = congenital adrenal hyperplasia; DHEAS = dehydroepiandrosterone sulfate; PCOS = polycystic ovary syndrome.

Source: See references 23 and 56.

23

MEN'S HEALTH

Mary Lee and Roohollah Sharifi

OBJECTIVES

After completing this chapter, the reader should be able to

- Develop a plan for monitoring testosterone supplementation for the treatment of late-onset hypogonadism
- Distinguish when serum free testosterone or bioavailable testosterone levels are preferred to serum total testosterone levels in selected patients
- Explain why minimal laboratory testing is used to evaluate a patient with new onset erectile dysfunction
- Make inferences about the presence or absence of voiding symptoms based on test results for peak urinary flow rate and postvoid residual urinary volume in patients with benign prostatic hyperplasia
- Argue for and against the use of prostate specific antigen screening for prostate cancer
- Explain the advantages and disadvantages of PCA3, TMPRSS2-ERG, and epigenetic testing over prostate specific antigen for patients with a total prostate specific antigen of 4–10 ng/dL
- Describe the alteration of prostate specific antigen levels in patients being treated with 5α-reductase inhibitors

(continued on page 594)

This chapter focuses on laboratory and clinical tests used to evaluate several common medical disorders in aging males—androgen deficiency, erectile dysfunction, benign prostatic hyperplasia (BPH), prostate cancer, and prostatitis. Tumor markers for assessing testicular cancer and laboratories for diagnosis of urinary tract infection and venereal diseases are discussed in other chapters.

HYPOGONADISM

Hypogonadism refers to medical conditions when the testes or ovaries fail to produce adequate amounts of testosterone or estrogen in men or women, respectively, to meet the physiologic needs of the patient. For the purposes of this chapter on men's health disorders, hypogonadism will refer to conditions when testicular production of testosterone is inadequate. Increasing patient age is associated with a greater percentage of men with serum testosterone levels that are below the normal range.[1] Out of approximately 900 men in the Baltimore Longitudinal Study, the calculated incidence of hypogonadism was 12%, 19%, 28%, and 49% in men in their fifth, sixth, seventh, and eighth decades of life, respectively.[2]

Testosterone Production and Physiologic Effects

Testosterone secretion is principally regulated by the hypothalamic-pituitary gonadal axis. The hypothalamus secretes gonadotropin-releasing hormone (GnRH). This acts on anterior pituitary receptors to stimulate the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH). FSH acts on testicular Sertoli cells to stimulate spermatogenesis, whereas LH stimulates testicular Leydig cells to produce testosterone. Once the serum level of testosterone increases into the normal physiological range, it triggers a negative feedback loop, which inhibits GnRH release from the hypothalamus. Pituitary LH release is inhibited too, but generally less so than GnRH.

Adrenocorticotrophin stimulates the adrenal gland to produce three androgens: dehydroepiandrosterone (DHA), dehydroepiandrosterone sulfate (DHAS), and androstenedione. DHA and DHAS are secreted at a daily rate of 15–30 mg, whereas androstenedione is secreted at a daily rate of 1.4 mg. All three adrenal androgens are weak androgens compared to testosterone. DHA and DHAS combined only contribute to 1% of circulating androgens, so the clinical effect of adrenal androgens is considered minor in normal men.

The principal androgen in males is *testosterone*. Testosterone comprises approximately 90% of circulating androgens. Testosterone enters the bloodstream and is delivered to target cells in muscle, bone, brain, reproductive and genital organs.[3] At some targets, testosterone itself appears to be physiologically active (e.g., central nervous system, bone, skeletal muscle, Sertoli cells). However, at other targets where 5α-reductase enzyme is expressed (e.g., prostate, scalp) testosterone is activated intracellularly by 5α-reductase to dihydrotestosterone (DHT), which has at least twice the potency of testosterone. Two separate forms of 5α-reductase enzymes exist: type I and type II. Each enzyme type tends to predominate in a particular tissue. Type I enzyme concentrates in the skin, liver, and sebaceous glands of the

OBJECTIVES

- **Describe the rationale for using age-related normal value ranges for prostate specific antigen, free prostate specific antigen, prostate specific antigen velocity, and prostate specific antigen density levels in evaluating patients with prostate cancer**
- **Explain the role of histologic Gleason scoring in managing patients with prostate cancer**
- **Contrast the 4-glass versus the 2-glass method for diagnosis of prostatitis**

scalp. Type II 5α-reductase predominates in the prostate and hair follicles of the scalp, and DHT in these tissues contributes to the development of BPH and alopecia, respectively.[3] Testosterone is responsible for various age-related physiologic effects in males, but most notably, it is responsible for development of secondary sexual characteristics in males (**Table 23-1**). In nontarget tissue, including the liver and adipose tissue, aromatase enzyme can convert excess androgen to estrone and estradiol.

In males, excess estrogen or a higher ratio of serum estrogen to androgen can result in gynecomastia and decreased libido. In young men, 4–10 mg of testosterone is produced each day. Testosterone secretion follows a circadian pattern, such that the highest secretion occurs at 7:00 a.m., and the lowest secretion occurs at 8:00 p.m. Testosterone circulates in three different forms: free (unbound) testosterone; bound to albumin or corticosteroid-binding globulin; or bound to sex hormone–binding globulin (SHBG). These forms comprise approximately 1–3%, 38–54%, and 44–60% of circulating testosterone levels, respectively. Free testosterone is physiologically active. Albumin-bound and corticosteroid-binding globulin-bound testosterone are inactive. However, testosterone can be easily released from these serum proteins, which have low affinity for the androgen. Therefore, this portion of testosterone has the potential to be bioavailable and become physiologically active.[4] Total bioavailable testosterone is about 50% of circulating serum testosterone. In contrast, SHBG has high affinity for testosterone, and SHBG-bound testosterone is physiologically inactive.

TABLE 23-1. Physiologic Effects of Testosterone and DHT[2-4]

STAGE OF LIFE OF MALE	PHYSIOLOGIC EFFECT
In utero	Normal differentiation of male internal and external genitalia
At puberty	Male body habitus, deepening of voice, male hair distribution, enlargement of testes, penis, scrotum, and prostate; increased sexual drive and bone growth
In adult	Sexual drive, muscle strength and mass, bone mass, prostate enlargement, male hair growth and distribution, spermatogenesis

DHT = dihydrotestosterone.

TABLE 23-2. Medical Conditions and Drugs That Alter SHBG Concentrations[5]

	INCREASED SHBG	DECREASED SHBG
Medical conditions that produce an alteration of SHBG concentration	Hepatic cirrhosis Hepatitis HIV disease Anorexia nervosa Hyperthyroidism Aging males Prolonged stress	Hypothyroidism Nephrotic syndrome Obesity Acromegaly Cushing syndrome Diabetes mellitus
Drugs that produce an alteration of SHBG concentration	Estrogens Phenytoin	Testosterone supplements, excessive doses Corticosteroids Progestins

HIV = human immunodeficiency virus; SHBG = sex hormone–binding globulin.

Multiple factors affect circulating SHBG level (**Table 23-2**). Measurement of SHBG levels is essential in patients when the serum total testosterone level is inconsistent with the clinical symptoms of the patient as it assists in the interpretation of the total serum testosterone level, and can be used to calculate the free or bioavailable testosterone level.

Hormonal Changes Associated with Primary, Secondary, and Tertiary Hypogonadism

Primary hypogonadism occurs when the testicles are absent or surgically removed, or when they are nonfunctional secondary to an acquired disease (e.g., mumps orchitis). *Secondary hypogonadism* occurs when the pituitary fails to release adequate amounts of LH; thus, the testes are not stimulated to produce adequate amounts of testosterone. *Tertiary hypogonadism* refers to a disorder of the hypothalamus such that there is inadequate release of GnRH, and a subsequent decrease in release of LH from the pituitary and testosterone from the testes (**Table 23-3**).

Late-Onset Hypogonadism

Late-onset hypogonadism, also known as *andropause* or *androgen deficiency in aging males* (ADAM), refers to the biochemical changes associated with age-related alterations in the hypothalamic-pituitary-gonadal axis, which may or may not be associated with clinically significant symptoms and signs (**Table 23-4**).[4,6] However, other men with decreased testosterone levels do not complain of their symptoms or have vague, nonspecific symptoms (e.g., malaise or decreased energy) for which they do not seek medical treatment.[6] Although late-onset hypogonadism is often compared to the menopause in aging females, these conditions are different (Table 23-4). In males gonadal function decreases over decades, and symptoms develop slowly and often are not attributed to decreasing hormone levels. In females, gonadal function decreases over a comparatively

TABLE 23-3. Etiology of and Laboratory Test Results in Patients with Primary, Secondary, and Tertiary Hypogonadism[5,6]

	PRIMARY	SECONDARY	TERTIARY
Common causes	Klinefelter syndrome Cryptorchidism Mumps orchitis Orchiectomy Irradiation of testes Traumatic injury to the testes 5α-reductase deficiency Noonan syndrome Autoimmune disorders (e.g., Hashimoto thyroiditis or Addison disease) Systemic disorders: HIV, hemochromatosis, cancer, liver cirrhosis High-dose radiation therapy Medications: high-dose ketoconazole, cytotoxins	Kallmann syndrome Pituitary adenoma or infarction Prolactinoma Sleep apnea Obesity Metabolic syndrome Type 2 diabetes mellitus Chronic renal failure Hepatic cirrhosis Hypothyroidism Anorexia nervosa Medications: estrogens, LHRH agonists (e.g., leuprolide, goserelin), LHRH antagonists (e.g., degarelix), ketoconazole, abiraterone, digoxin, prolonged course of high-dose corticosteroids, megestrol acetate, medroxyprogesterone, long-acting opioids	Infectious or infiltrative diseases of the hypothalamus (e.g., tuberculosis, sarcoidosis, infectious abscess) Isolated gonadotropin deficiency
Serum testosterone level	Decreased	Decreased	Decreased
LH level	Increased	Decreased	Decreased
GnRH level	Increased	Increased	Decreased

GnRH = gonadotropin-releasing hormone; HIV = human immunodeficiency virus; LH = luteinizing hormone; LHRH = luteinizing hormone–releasing hormone.

TABLE 23-4. Characteristics of Late-Onset Hypogonadism in Aging Males Versus Menopause in Aging Females[7]

	LATE-ONSET HYPOGONADISM	MENOPAUSE
Time period over which gonadal function decreases	Decades, beginning at age 30–40 yr[3]	4–6 yr, beginning approximately at age 50–52 yr
Fertility is maintained	Yes	No
Symptoms and signs	Decreased libido, erectile dysfunction, gynecomastia, weight gain, visceral obesity, moodiness, decreased sense of well-being, muscle aches, decreased muscle mass and strength (sarcopenia), weight gain, osteopenia, osteoporosis, hot flashes, reduced body hair, decreased testicular size, infertility	Menstrual cycles become progressively heavier and lighter, shorter and longer, and then stop; hot flashes, weight gain, vaginal dryness, dyspareunia, and hair loss
Symptoms and signs are linked to serum level of gonadal hormone	No	Yes

shorter time period of four to six years, and symptoms are closely associated with decreasing hormone levels.

Hormonal Changes Associated with Late-Onset Hypogonadism

Starting at age 40, serum testosterone levels decrease by 1–2% annually. At age 80, the mean serum testosterone declines by approximately 40% of that typically observed in men at age 40.[10] Low serum testosterone levels in patients with late-onset hypogonadism are due to multiple physiologic changes including the following[4,6]:

- Increased sensitivity of the hypothalamus and pituitary gland to negative feedback; thus, even low circulating testosterone levels stimulate the negative feedback loop
- Irregular, nonpulsatile secretion pattern of LH
- A smaller difference in peak and trough serum concentrations during the day when compared to young adult males[8,9]
- Increased production of SHBG, which increases the plasma concentration of physiologically inactive SHBG-bound testosterone
- Decreased number of functioning Leydig cells, which results in an age-related decrease in testicular production of testosterone

Of importance, late-onset hypogonadism was once thought to be a type of primary hypogonadism. However, multiple alterations in the hypothalamic pituitary gonadal axis suggest that late-onset hypogonadism is a mixed type of hypogonadism. This phenomenon occurs in the face of a wide range of serum (total) testosterone and bioavailable testosterone levels among elderly males.[5] Whereas some symptomatic elderly males have serum testosterone levels that are below the normal physiological range, others have levels that are decreased but are still within the normal range.[10]

A male patient, age 50 years or older, who presents with symptoms or signs of hypogonadism should undergo laboratory evaluation for late-onset hypogonadism. However, this is a diagnosis of exclusion, which is made after all other causes of low serum concentrations of testosterone have been ruled out. To assess symptom severity, the patient is commonly asked to complete a validated self-assessment questionnaire at baseline and at regular intervals after treatment is started. Because of the lack of specificity, these questionnaires are used to monitor a patient's treatment response, as opposed to disease diagnosis. For example, the St. Louis University Androgen Deficiency in Aging Males (ADAM) questionnaire includes 10 questions, which can be categorized into three symptom domains (psychologic, somatovegetative, and sexual), and to which the patient responds either "yes" or "no."[11,12] An affirmative response to at least three questions on the survey is considered significant. The ADAM questionnaire has a sensitivity of 88% and a specificity of 60%.

A similar alternative self-assessment instrument is the Aging Males' Symptoms (AMS) scale, which has a sensitivity of 83% and a specificity of 39%[13] The AMS questionnaire comprises 17 questions focused on symptoms associated with low testosterone. The patient rates the severity of each symptom on a scale of 1 (none) to 5 (extremely severe). Finally, a third tool, the Massachusetts Male Aging Survey (MMAS) has a sensitivity of 60% and a specificity of 59%. It is comprised of eight, multiple-choice questions. Each response is assigned a certain number of points. Once all of the points are tallied, the total score has implications about the likelihood that the patient has hypogonadism. For example a total score of 10 or higher suggests that the patient has a 50-50 chance that he has hypogonadism, whereas a score of 4 or below suggests that the patient probably does not have hypogonadism. The MMAS is considered the least sensitive of the three tools.[11]

Late-onset hypogonadism should be treated with testosterone supplements if the patient has both symptoms of hypogonadism and an unequivocal serum testosterone level of 200–230 ng/dL or less, based on at least two separate serum testosterone measurements on different days, and provided that the patient has no contraindications for androgen supplementation.[5,6,14] In addition to relieving symptoms and potentially preventing osteoporosis and bone fracture from long-term hypogonadism, treatment may decrease the prevalence of prostate cancer, which tends to occur more frequently in men with hypogonadism.[15] For a patient with symptoms of hypogonadism and a serum testosterone level of 250–360 ng/dL, the treatment of late-onset hypogonadism should be decided after assessing the benefits versus the risks of androgen supplementation by the physician and patient. Controversy exists on the value of testosterone replacement in this subset of patients.[5,14] As long as the serum testosterone level is in the normal physiologic range, some physicians will not prescribe testosterone supplements. This is because some evidence suggests that administration of exogenous androgens to increase the serum testosterone level from one end of the normal range to a higher point within the normal range does not improve or increase sexual drive or energy.[16,17] Rather than prescribe testosterone supplements, the physician will treat individual symptoms with specific nonandrogen treatments. For example, if the patient is moody or depressed, psychotherapy may be beneficial. If the patient has erectile dysfunction, then a phosphodiesterase inhibitor may be indicated. On the other hand, there is evidence that suggests that signs and symptoms of hypogonadism respond to different levels of serum testosterone; libido may be restored at the lower end of the normal physiologic range, whereas increased bone mineralization is observed when serum testosterone is at the higher end of the normal physiologic range.[4,17] For this reason, some physicians will prescribe testosterone replacement to patients who have serum testosterone levels at the low end of the normal range. Patients with symptoms of hypogonadism and a serum testosterone level of 350–360 ng/dL or more should not receive testosterone supplements, unless the free or bioavailable testosterone levels are low.[14]

Once a testosterone replacement regimen is initiated, the patient should return for assessment of the efficacy and safety of treatment every three or four months during the first year.[14,17,18] Once the serum testosterone increases to the mid-normal range (400–700 ng/dL), serum levels should be repeated annually thereafter.[18] A minimum clinical trial of a testosterone supplement is three months.[4,6,18]

Other assessments include a persistent reduction of symptoms of hypogonadism as assessed by medical history and the patient's responses to a validated self-assessment questionnaire (e.g., ADAM, AMS, or MMAS). Because testosterone can theoretically stimulate prostate enlargement and is a cocarcinogen in the development of prostate cancer, the patient should annually undergo a digital rectal exam of the prostate and PSA testing. Finally, because excess androgen is converted to estrogen, which can induce breast cancer or gynecomastia, the patient should undergo breast examination prior to the start of and periodically during treatment.

The onset of symptomatic improvement after initiating a testosterone replacement regimen is variable. An increase in libido and an improved perception of quality of life is evident in three weeks; signs of decreased mental depression may first appear at three to six weeks; increased muscle strength and decreased fat mass are observable at 12 to 16 weeks; and improved erectile dysfunction and bone density occur at six months.[19]

Testosterone, Total

Normal range, adult male: 280–1100 ng/dL
(9.7–38.17 nmol/L)
Normal range, age-related:
Male, 6–9 yr old: 3–30 ng/dL (0.1–1.04 nmol/L);
Male, pubertal: 265–800 ng/dL (9.2–27.76 nmol/L)

A routine serum testosterone level reflects the total concentration of testosterone in the bloodstream, in all three of its forms: free, bound to albumin or corticosteroid-binding globulin, and bound to SHBG. Testosterone secretion follows a circadian pattern in younger males such that morning levels are approximately 15–20% (but can range up to 50%) higher than evening levels, which is a difference of approximately 140 ng/dL between the peak and nadir serum levels.[5] In addition, variability in measured testosterone levels is characteristic from day to day, from week to week, and seasonally.[5] This can result in a 10% difference in measured serum testosterone levels from the same patient.[5] Thus, when obtaining serum testosterone levels, it is recommended that blood samples be obtained between 8:00 a.m. and 11:00 a.m. Furthermore, to confirm a low serum testosterone level, it also is recommended that a second sample be obtained usually at least one week apart. This is necessary because 30% of men with initial serum testosterone levels that are in the hypogonadal range will have levels in the normal range when the laboratory test is repeated.[20] If the patient has a medical disorder or is taking medication that can alter serum testosterone levels, it is recommended that testing for serum testosterone levels be deferred until the medical disorder resolves or the medication is discontinued. Common causes of decreased (Table 23-3) and increased serum testosterone levels are listed in **Table 23-5**.

The normal range is wide for serum testosterone levels and is based on laboratory results for young adult males. Although this normal range is applied to interpretation of serum testosterone levels in elderly males, no single threshold serum testosterone value has been identified to be pathognomonic for hypogonadism or low serum testosterone in this age group.[5,17,18,21] As an example, the Food and Drug Administration defines hypogonadism using a serum testosterone of <300 ng/dL as an inclusion criteria for patients in controlled clinical trials.[5] However, some clinicians view that using a threshold serum testosterone value of <300 ng/dL will result in excessive, unnecessary treatment of hypogonadism in asymptomatic men. As a result, the International Society for the Study of the Aging Male, the International Society of Andrology, the European Association of Urology, the European Association of Andrology, and the American Society of Andrology collectively have taken the position that a serum testosterone level of <230 ng/dL may be treated if the patient is symptomatic, but a serum testosterone level >350 ng/dL generally should not be associated with symptoms, nor should it be treated.[14] Generally, the following interpretation of serum testosterone levels is used:

- Serum testosterone <200 ng/dL—Most likely the patient has symptomatic hypogonadism and will require testosterone replacement.
- Serum testosterone 250–360 ng/dL—This level is equivocal for hypogonadism. The patient should be treated only if a low serum testosterone, free testosterone, or bioavailable testosterone level is confirmed, and if the patient is symptomatic. (**Minicase 1.**)
- Serum testosterone >600 ng/dL—The patient does not have hypogonadism and requires no testosterone replacement.
- After instituting a testosterone replacement regimen, the goal of treatment is to increase the serum testosterone level to 400–700 ng/dL.[22]
- When treating patients with prostate cancer with LHRH agonists or antagonists, medical castration is induced. The target serum testosterone level is ≤50 ng/dL.

Testosterone levels are commonly determined using radioimmunoassay, nonradioactive immunoassays, or chemiluminescent detection methods, which are relatively easy to perform and inexpensive. However, these methods exhibit significant performance variability in the normal range and are limited in detecting serum testosterone levels <300 ng/dL.[5,23,24] That is, these assays may produce results that are significantly different than the true value.[5] Thus, it is recommended that a physician try to ensure that a single patient's serum testosterone levels be assayed by the same clinical laboratory over time and that a normal range of serum testosterone be determined for each clinical laboratory that runs the assay.[25] Such a determination would require measurement of serum testosterone in approximately 40 normal, healthy men, age 20–40 years.[4,7] The Centers for Disease Control and Prevention has initiated a program to standardize testosterone assays, which involves providing reference material to calibrate immunoassays. This should reduce the variability of testosterone laboratory results among laboratories.[24] Despite the availability of more sensitive and specific antibodies for radioimmunoassays, stable isotope dilution liquid chromatography using benchtop tandem mass spectrometry (LC-MS) is generally considered the gold standard method for assaying serum testosterone. LC-MS has improved accuracy and precision over radioimmunoassay and can measure serum testosterone levels <300 ng/dL.[5,26]

TABLE 23-5. Common Causes of Increased Total Testosterone Levels[5,17]

INCREASED
Hyperthyroidism
Adrenal tumors
Adrenal hyperplasia
Testicular tumors
Precocious puberty
Excessive exogenous testosterone use (usually seen with parenteral, but not transdermal use)
Anabolic steroids

Free Testosterone

Normal age-related range, adult male:
10–15 ng/dL, age 20–29 yr
9–13 ng/dL, age 30–39 yr
7–11 ng/dL, age 40–49 yr
6–10 ng/dL, age 50–59 yr
5–9 ng/dL, age >60 yr

Free testosterone levels are the best reflection of physiologically active androgen. When compared to young adult males, elderly

MINICASE 1

A Patient with Erectile Dysfunction and Normal Serum Testosterone Levels

John A. is a 60-year-old, white male who complains of erectile dysfunction and no sexual drive. He feels like he is disappointing his sexual partner because he has no desire for sexual intercourse and cannot seem to perform adequately. He attributes all of this to getting older and wonders if a pill can "make him better."

Cc: John A. has erectile dysfunction and decreased libido.

HPI: John A. reports that the problem has been getting worse over the past two years. Initially, he had periodic erectile dysfunction. Now, he has no nocturnal erections and can't get an erection when he needs it. He still has morning erections.

PMH: Essential hypertension and hypercholesterolemia

Medications: Hydrochlorothiazide, valsartan, and pravastatin

Allergies: None

Physical exam:

ROS: Well-developed, well-nourished male with mild nocturia and some urinary hesitancy, particularly in the morning

Vital signs: BP 140/87 mg Hg, HR 65 beats/min, RR 16 breaths/min, temperature 98.6 °F, weight 75 kg, height 5'11"

Genitourinary tract: Normal penis, no curvature; testes, mildly atrophic; anal sphincter tone intact and within normal limits; digital rectal exam reveals mildly enlarged prostate; pedal pulses, PSA 1.2 ng/mL

Assessment: Suspect late-onset hypogonadism with erectile dysfunction and BPH

QUESTION: A serum total testosterone level is ordered. The result is 350 ng/dL. Is testosterone supplementation indicated?

DISCUSSION: The patient's decreased libido and erectile dysfunction are consistent with late-onset hypogonadism. However, his serum testosterone is at the low-end of the normal range. Because of high intrapatient variability in measured serum testosterone levels, a repeat serum testosterone level should be obtained before initiating testosterone supplementation.

QUESTION: The second serum testosterone level is 325 ng/dL, and the patient is still complaining of no sexual drive and erectile dysfunction. Is testosterone supplementation indicated?

DISCUSSION: The second serum testosterone level is in the equivocal range. It would be useful to measure sex hormone–binding globulin levels and to calculate the free or bioavailable testosterone levels to assess the adequacy of physiologic testosterone levels in this patient before testosterone supplementation is initiated. This patient's erectile dysfunction may not be due to hypogonadism. It will likely respond to a phosphodiesterase inhibitor.

males experience an almost 20% decrease in albumin-bound testosterone and an almost 20% increase in SHBG-bound testosterone in the circulation. Because free testosterone is in equilibrium with testosterone bound to albumin or corticosteroid-binding globulin, the amount of bioavailable testosterone is decreased in elderly males; therefore, elderly males may develop symptoms of hypogonadism despite having serum total testosterone levels near the normal range (**Table 23-6**).

Free testosterone levels are altered by the concentration of SHBG. Thus, free testosterone levels are preferred to assess testosterone adequacy in patients with diseases or taking medications, which increase or decrease levels of SHBG (Table 23-2), or when the patient has symptoms of hypogonadism but has a serum total testosterone in the equivocal range.[5,18] A free testosterone level that is <6.5 ng/dL in the presence of symptoms of hypogonadism strongly suggests that the patient would benefit from testosterone replacement.[14]

Free testosterone levels are subject to the same diurnal patterns of total testosterone levels. Therefore, samples should be drawn in the morning.[5] The most accurate assay method to measure free testosterone is by centrifugal ultrafiltration or equilibrium dialysis technique. However, such assays are not routinely available, are time-consuming, and are expensive. Thus, many laboratories offer radioimmunoassay for free testosterone levels. Although inexpensive, this method is associated with less accurate results.[27] Saliva specimens using a direct luminescence immunoassay can be used to measure free testosterone but is rarely done.[28]

If free testosterone levels cannot be measured using an assay, the level may be estimated (a commonly used calculator is available at http://www.issam.ch/freetesto.htm). By inserting the values for measured serum levels of albumin, SHBG, and total testosterone into the online calculator, the patient's free testosterone level is derived. Estimated values are comparable to measured values by equilibrium dialysis.[19,29]

TABLE 23-6. Comparison of the Percentage of Serum Bioavailable Testosterone in Young Versus Old Male[7]

	YOUNG MALE	OLDER MALE
% of total testosterone, which is free testosterone	2	2
% of total testosterone, which is bound to albumin	38	20
% of total testosterone, which is bound to SHBG	60	78
% of bioavailable testosterone (% free + % albumin-bound)	40	22

SHBG = sex hormone–binding globulin.

Bioavailable Testosterone

Normal age-related range, adult male:
83–257 ng/dL, age 20–29 years
72–235 ng/dL, age 30–39 years
61–213 ng/dL, age 40–49 years
50–190 ng/dL, age 50–59 years
40–168 ng/dL, age 60–69 years
Not established, age >70 years
Also expressed as percentage of total serum testosterone
Normal range, adult male: 12.3–63%

Bioavailable testosterone levels measure the concentration of free testosterone and albumin-bound testosterone in a serum sample. Because albumin has low affinity for testosterone, reversible binding of testosterone to albumin allows an equilibrium to be established between free and albumin-bound testosterone fractions. Thus, these two forms of circulating testosterone are considered bioavailable and physiologically active.[4,7] As men age, bioavailable testosterone levels decrease as serum SHBG levels increase. Similar to free testosterone levels, which are dependent on SHBG levels, bioavailable testosterone levels may be preferred when assessing testosterone activity in patients with significant alterations of SHBG (Table 23-2 and Table 23-6).

An ammonium sulfate precipitation assay is used to measure bioavailable testosterone. It is expensive and technically challenging to perform. For this reason, this test is not commonly available in clinical laboratories. If bioavailable testosterone levels cannot be measured using an assay, the level may be estimated (a commonly used calculator is available at http://www.issam.ch/freetesto.htm). By inserting the values for measured serum levels of albumin, SHBG, and total testosterone into the online calculator, the patient's bioavailable testosterone level is derived.

ERECTILE DYSFUNCTION

Erectile dysfunction is the consistent inability over a minimum duration of three months to achieve a penile erection sufficient for sexual intercourse.[30] The prevalence of erectile dysfunction increases with increasing patient age. According to the Massachusetts Male Aging Study, the prevalence of moderate erectile dysfunction increases in men from the 4th decade of life to the 6th decade of life, from 12–46%, respectively.[31] In the health professional study of men, age 50 years or older, the overall prevalence of erectile dysfunction was 33%, with an increased prevalence in patients with risk factors, including cigarette smoking, excessive alcohol intake, sedentary lifestyles, and obesity.[32,33] However, advancing age is not considered an independent risk factor for erectile dysfunction.

The causes of erectile dysfunction are broadly divided into two types: *organic* and *psychogenic*.[30,34] Most patients with erectile dysfunction have the organic type, in which concurrent medical illnesses interfere with one or more physiologic components essential for a penile erection (**Table 23-7**).[34] That is, the patient has one or more medical illnesses that impairs vascular flow to the corpora cavernosa; impairs central or peripheral innervation necessary for a penile erection; or is associated with testosterone insufficiency, in which case the patient develops erectile dysfunction secondary to a decreased libido. Psychogenic erectile dysfunction is commonly situational in that the patient is unable to have an erection with a particular person, has performance anxiety, or is recovering from a major life stress (e.g., loss of a job, divorce, death in the family).[34]

Because current first choice treatment for erectile dysfunction is effective in up to 70% of treated patients independent of the etiology, the diagnostic assessment of these patients has been streamlined. These patients are commonly diagnosed in primary care clinics with a comprehensive sexual history to identify the particular type of sexual dysfunction that the patient has (e.g., decreased libido, erectile dysfunction, or ejaculation disorder). Details on onset of symptoms are obtained along with a patient's self-assessment of the severity of the problem using a validated, reliable questionnaire (e.g., International Index of Erectile Function), and information on the patient's expectations for improved sexual function from the patient and from the spouse or significant other.[35,36]

A comprehensive medical history is then performed to identify treatable underlying diseases, which may be contributing

TABLE 23-7. Comparison of Organic and Psychogenic Erectile Dysfunction[30]

	ORGANIC	PSYCHOGENIC
Patient age	Older male	Younger male
Onset	Gradual, unless erectile dysfunction is due to traumatic injury	Sudden and complete loss of erectile function
Linked to a particular event in the patient's life	No	Yes (e.g., divorce, job-related stress financial stress)
Patient has a normal libido	Yes	No
Patient has nocturnal erections, which are reflex reactions	No	Yes
Patient has erections on awakening	No	Yes
Patient has erections with masturbation	No	Yes
Patient has erections with foreplay	No	Yes
Patient has concurrent medical illnesses that could contribute to erectile dysfunction	Yes	No
Patient's partner is perceived to be a problem in the relationship prior to the onset of erectile dysfunction	No	Yes
Patient has performance anxiety prior to the onset of erectile dysfunction	No	Yes

TABLE 23-8. Common Causes of Organic Erectile Dysfunction[30,34]

IMPAIRMENT	HOW IT CAUSES ERECTILE DYSFUNCTION	EXAMPLE DISEASES/CONDITIONS ASSOCIATED WITH THIS TYPE OF IMPAIRMENT
Vascular	Decreased arterial flow to the corpora cavernosa	Hypertension Congestive heart failure Coronary artery disease Arteriosclerosis Smoking Obesity Peripheral vascular disease Chronic, heavy smoking Drugs that cause hypotension: antihypertensives, central and peripheral sympatholytic agents
Neurologic	Decreased central processing of sexual stimuli or impaired peripheral nerve transmission, which decreases erectogenic reflex responses to tactile stimuli	Stroke Diabetes mellitus Chronic alcoholism Postradical prostatectomy in which pelvic nerve injury has occurred Epilepsy Multiple sclerosis Parkinson disease Psychosis Major depression Pelvic trauma with nerve injury Spinal cord injury Drugs with anticholinergic effects: antispasmodics, phenothiazines, tricyclic antidepressants, first-generation antihistamines, etc.
Hormonal	Decreased serum testosterone, increased serum estrogen, increased ratio of serum estrogen to serum testosterone, or hyperprolactinemia results in decreased libido; erectile dysfunction is secondary to the decrease in libido	Late-onset hypogonadism Primary or secondary hypogonadism Hypothyroidism/hyperthyroidism Hyperprolactinemia Adrenal gland disorders Drugs with estrogenic effects or that decrease androgen production or action: diethylstilbestrol, LHRH superagonists, LHRH antagonists
Anatomic	Penile deformity or curvature when erect	Peyronie disease Traumatic injury to the penis

LHRH = luteinizing hormone–releasing hormone.

to erectile dysfunction (**Table 23-8**).[30] In addition, because erectile dysfunction may be the first presenting symptom of underlying cardiovascular or metabolic diseases, the clinician will investigate thoroughly for such conditions.[37] For example, blood pressure is measured and, if elevated, is treated. The patient is instructed to discontinue smoking, if applicable. A physical exam is completed to check for signs of hypogonadism. Peripheral pulses are palpated to assess vascular integrity. A thorough urological examination to evaluate the integrity of the lower urinary tract and functional status of the bladder, urethra, and external genitalia is mandatory. A digital rectal exam is conducted on patients who are 50 years of age or older. This checks for anal sphincter tone, which indicates adequacy of sacral nerve innervation to the corpora cavernosa; prostate enlargement, which could obstruct urinary flow and lead to incontinence (which has been linked to erectile dysfunction); and a nodular or indurated prostate, which is suggestive of prostate cancer. Finally, an examination of the external genitalia identifies the presence of penile deformity or tissue scarring, which may contribute to erectile dysfunction.

For patients age 50 years or older who have a life expectancy of at least 10 years, a blood test for prostate specific antigen (PSA) is obtained. If the medical or medication history suggests that the patient has concurrent medical illnesses that may contribute to erectile dysfunction, laboratory tests should be obtained to determine if these medical illnesses require more aggressive treatment. Such laboratory tests include a fasting blood glucose for diabetes mellitus, a lipid profile for hypercholesterolemia, a

TABLE 23-9. Specialized Diagnostic Testing for Erectile Dysfunction[30,34]

TEST	DESCRIPTION	PURPOSE OF TEST
CIS testing	A single dose of alprostadil, papaverine, and phentolamine is administered as an intracavernosal injection	Allows visual assessment of vascular integrity of penile arterial and venous flow
Duplex ultrasonography	CIS is performed, then ultrasound and Doppler imaging of arterial flow to the corpora cavernosa is done	Allows assessment of the flow through the main dorsal artery and the cavernous artery

CIS = combined intracavernous injection and stimulation.

urinalysis to check for genitourinary tract disorders, serum testosterone levels for hypogonadism, a serum prolactin level if the patient has erectile dysfunction, decreased libido, and gynecomastia. Specialized clinical testing is reserved for patients prior to surgical correction of erectile dysfunction in patients who do not respond to drug therapy or noninvasive medical devices, including oral phosphodiesterase inhibitors (e.g., sildenafil), intracavernosal alprostadil, intraurethral alprostadil inserts, and vacuum erection devices (**Table 23-9**).

International Index of Erectile Function, Sexual Health Inventory for Men, and Brief Male Sexual Function Inventory

The *International Index of Erectile Function* (IIEF) is a validated self-assessment questionnaire that includes 15 questions. The patient assesses the presence and severity of decreased libido, erectile or ejaculatory dysfunction, diminished orgasm, and his overall satisfaction with his sexual performance for the past month.[35] The questionnaire takes approximately 10–15 minutes to complete. Total scores for each domain can be calculated; and each of these scores is associated with severity level (**Table 23-10** and **Table 23-11**). The IIEF is used at baseline to assist the physician in determining the severity of erectile dysfunction. Once treatment is initiated, the patient is asked to complete the IIEF questionnaire again so that the physician can assess the level of improvement in erectile function.

TABLE 23-10. Domains and Maximum Score for Each Domain of the IIEF

	NUMBER OF QUESTIONS RELATED TO THE DOMAIN	RANGE OF SCORES FOR QUESTION	MAXIMUM TOTAL SCORE FOR THE DOMAIN
Erectile function	6	0–5	30
Orgasmic function	2	0–5	10
Sexual desire	2	0–5	10
Intercourse satisfaction	3	0–5	15
Overall satisfaction	2	0–5	10

IIEF = International Index of Erectile Function.

A shorter self-assessment questionnaire is an abridged IIEF, which includes five of the 15 questions from the original survey that focus on erectile dysfunction and the last question concerning the patient's overall satisfaction with his sexual performance. This is known as the Sexual Heath Inventory for Men.[36] Some clinicians consider this shorter questionnaire to be more practical to use than the original IIEF. Another commonly used tool is the Brief Male Sexual Function Inventory. Table 23-11 summarizes the differences among these three self-assessment questionnaires.

Prolactin

Normal range, adult males: 0–15 ng/mL or 0–15 mcg/L (0–652 pmol/L)

Prolactin is secreted by the lactotroph cells of anterior pituitary gland in multiple pulses during the day. The normal daily production rate is 200–536 mcg per m^2 total body surface area.

TABLE 23-11. Summary of Self-Assessment Tools for Patients with Erectile Dysfunction

	INTERNATIONAL INDEX OF ERECTILE FUNCTION	SEXUAL HEALTH INVENTORY FOR MEN	BRIEF MALE SEXUAL FUNCTION INVENTORY
Number of questions in the tool	15	5	11
Focus of questions	Sexual desire, erectile function, orgasm, satisfaction with sexual intercourse, overall satisfaction	Erectile dysfunction	Sexual drive, erection, ejaculation, perception of problems with sexual function, overall satisfaction
Assessment scale	Each of the five focus areas can be individually assessed based on the specific questions answered by the patient; for example, for erectile function, there are six questions; the score for each item ranges from 0 to 5; a total score of 0–6 implies severe dysfunction, 7–12 implies moderate dysfunction, 13–18 implies mild-to-moderate dysfunction, 19–24 implies mild dysfunction, and 25–30 implies no dysfunction	Each item is rated on a 5-point scale; score for each item ranges from 1 to 5; total score for the survey characterizes the severity of erectile dysfunction, such that 22–25 equals no dysfunction, 17–21 equals mild dysfunction, 12–16 equals mild-to-moderate dysfunction, 8–11 equals moderate dysfunction, and 5–7 equals severe dysfunction	Each item is rated on a 5-point scale; score for each item ranges from 0 to 4; each score has an individualized anchor descriptor

Although some prolactin circulates in inactive dimeric form (also known as *big prolactin*) or in a less active form complexed to IgG immunoglobulin (also known as *big, big prolactin* or *macroprolactin*), 85–95% exists as monomeric prolactin, which is physiologically active hormone.[38] Its pulsatile secretion is predominately controlled by prolactin inhibitory factor, which is thought to be a dopamine$_2$-like substance secreted by the hypothalamus in response to high levels of prolactin in the systemic or hypophyseal portal circulation. A prolactin stimulatory factor also may regulate prolactin secretion; however, its chemical structure still needs to be identified. It may be similar to vasoactive intestinal polypeptide or thyrotropin-releasing hormone, which both simulate prolactin secretion.[39] Prolactin follows a diurnal pattern of secretion with highest serum levels occurring when the patient sleeps at night. Nadir levels occur between 10:00 a.m. and 12:00 p.m. The precise role of prolactin in males is unclear; however, it has been hypothesized that high circulating prolactin levels suppress LH and FSH, thereby decreasing testosterone production, decreasing semen volume and spermatogenesis.[40] It also is known that hyperprolactinemia decreases libido. Prolactin is excreted renally.

True hyperprolactinemia occurs in 1–2% of men who present with decreased libido, erectile dysfunction, and gynecomastia, and is characterized by the presence of high levels of monomeric prolactin. Medical conditions and medications that can produce hyperprolactinemia are included in **Table 23-12**. They can be broadly classified as disorders of the hypothalamus or pituitary gland, neoplastic conditions, metabolic disorders, or medication-related causes. Whereas hypothalamic (e.g., craniopharyngioma), pituitary (e.g., prolactinoma), and paraneoplastic syndromes can cause significant increases in serum prolactin levels exceeding 250 ng/mL, systemic diseases, medications, sleep, pain, or meals cause smaller increases in serum prolactin level that rarely exceed 200 ng/mL.[41-46] It should be noted that decreased prolactin levels in a male is a rare condition. The clinical significance of this finding is unknown as it is associated with no symptoms or disease.

Indications for assessing serum prolactin levels include (1) a patient who is <50 years of age and complains of decreased libido, erectile dysfunction, infertility, and gynecomastia, or who has low serum testosterone levels; (2) a patient who is >50 years of age and complains of gynecomastia; (3) a patient with late-onset hypogonadism and erectile dysfunction, whose symptoms are not corrected with a testosterone replacement regimen; or (4) a patient with symptoms consistent with prolactinoma (i.e., persistent headache, cranial nerve palsies, and visual field defects). A diagnosis of hyperprolactinemia generally requires documentation of two elevated prolactin levels.[42]

Prolactin levels should not be routinely obtained in patients who present with erectile dysfunction.

Assay techniques for prolactin measurement include immunoassays using chemiluminescent, fluorescent, or radioactive labels. To minimize interference of prolactin assays by meals and stress, both of which can increase prolactin levels, it is recommended that blood specimens be collected three or four hours after the patient has awakened and fasted overnight. Prior to the blood draw, it is recommended that the patient rest for at least 20–30 minutes.[39]

TABLE 23-12. Medical Conditions and Medications Associated with Increased or Decreased Prolactin Levels[41-47]

INCREASED PROLACTIN LEVELS	DECREASED PROLACTIN LEVELS
Pituitary adenoma (nonprolactinoma)	Panhypopituitarism
Pituitary prolactinoma	Pituitary infarction
Acromegaly	Extended critical illness
Severe head trauma	Medications: carbamazepine, phenytoin, valproic acid, bromocriptine, clonidine, ergot alkaloids, levodopa, pergolide, nifedipine, rifampin, tamoxifen
Craniopharyngioma	
Paraneoplastic syndrome with ectopic production of prolactin	
Primary hypothyroidism	
Renal failure, chronic	
Liver cirrhosis	
Addison disease	
Idiopathic pituitary hyperprolactinemia	
Stress, provoked by surgery, hypoglycemia, myocardial infarction	
Sarcoidosis	
Chest wall trauma	
Seizures	
Epilepsy	
Anorexia nervosa	
Sexual intercourse	
Medications: phenothiazines, butyrophenones, thioxanthenes, buspirone, olanzapine, risperidone, haloperidol, loxapine, pimozide, tricyclic antidepressants, selective serotonin reuptake inhibitors, molindone, quetiapine, monoamine oxidase inhibitors, oral contraceptives, estrogens, megestrol, opiates, methadone, cocaine, tetrahydrocannabinol, antihistamines, ranitidine, cimetidine, metoclopramide, pimozide, reserpine, methyldopa, verapamil, labetalol, phenytoin	

Big prolactin and big, big prolactin (macroprolactin), which are the less active or inactive forms of prolactin, cross react with prolactin in immunoassays.[48] Macroprolactinemia is diagnosed when the majority of the circulating prolactin is of the macroprolactin form.[49] Separating patients with macroprolactinemia from those with true hyperprolactinemia is important to avoid expensive diagnostic testing and unnecessary or invasive treatment of the former, which is a benign disease.[48] To distinguish macroprolactin from prolactin, polyethylene glycol extraction and centrifugal ultrafiltration assay methods can be employed.[52] Although simple to perform, the assay method is sensitive but not specific for macroprolactin. Gel filtration chromatography is considered the gold standard assay.[38,48,49]

Extremely high serum levels of prolactin may saturate the ability of immunoassays to measure correct levels. This is known as the *hook effect*. Therefore, in patients with prolactinomas, it may be necessary to dilute the specimen to 1:100 before assaying.[39,41,49,50]

BENIGN PROSTATIC HYPERPLASIA

Benign prostatic hyperplasia (BPH) is an enlargement of the prostate gland that occurs in all males as they age. The histologic disease prevalence is 80% in men age 70–79 years.[51] Furthermore, 50% of men with a histologic diagnosis of BPH develop clinical symptoms of at least moderate severity.[52] Beginning at approximately age 40 years in males, the prostate gland undergoes a second growth spurt, which is stimulated by DHT, and the prostate grows from a normal adult size of 15–20 g to a much larger size that can exceed 100 g. The local complications of BPH include obstructive and irritative voiding symptoms. Collectively, these symptoms are often referred to as *lower urinary tract symptoms* (LUTS); however, they are not specific for BPH and may be due to other genitourinary tract disorders (e.g., neurogenic bladder, prostate cancer, urethral stricture, prostatitis, and urinary tract infection).[52] Obstructive symptoms include a slow urinary stream, difficulty emptying urine out of the bladder, hesitancy, dribbling, a sensation of incomplete bladder emptying, and straining to void. Such symptoms can be due to the enlarged prostate, which produces an anatomical block of the bladder neck. Irritative symptoms include urinary frequency, nocturia, and urgency, which may progress to urinary incontinence. Such symptoms are due to the long-term effects of obstruction on the detrusor muscle of the bladder. That is, an enlarged prostate results in partial obstruction of the bladder outlet. In time, this causes hypertrophy of the bladder muscle and increased intravesical pressure, which translates to urgency, frequency, nocturia, and urge incontinence. If untreated, progressive increased resistance at the bladder outlet will result in decompensation and residual urine, and then total urinary retention and overflow incontinence. Other complications of untreated, severe BPH include recurrent urinary tract infection, urosepsis, recurrent or intermittent gross hematuria, urolithiasis (primarily bladder stones), and chronic renal failure.

The symptoms of BPH are most often the driver that brings the patient to medical attention. Nocturia, which interferes with sleeping, and urgency-associated incontinence, which curbs social activity, can significantly reduce quality of life. Thus symptom assessment is crucial in evaluating the disorder. Symptom assessment is typically completed by having the patient use a validated questionnaire such as the American Urological Association Symptom Index (AUA-SI) score or the International Prostate Symptom Score (IPSS).[52,53]

Signs of disease are evaluated by the physician using clinical procedures that can be performed easily in an outpatient setting. A careful medical history is taken to identify any concurrent medical illnesses or medications that may be causing LUTS or worsening LUTS. A physical examination should be performed to check for bladder distention and neurologic innervation of the lower urinary tract. A digital rectal exam is performed to assess prostate gland size, shape, and consistency, and anal sphincter tone. The latter is innervated by branches of the pudendal nerve, which emanates from the sacral plexus at S2-S4, and also is responsible for bladder contraction and emptying. In addition, to rule out other common causes of urinary frequency and urgency, physicians should obtain a urinalysis. Microscopic examination of the spun sediment for white blood cells (WBCs) and bacteria, and a dipstick check for leukocyte esterase and nitrite help identify urinary tract infection as a cause for the patient's symptoms. If gross or microscopic hematuria is present and the patient has a past or current history of smoking, urine is sent for cytological assessment. Bladder neoplasms typically shed cancer cells into the urine. For patients in whom the urinalysis is suspicious for renal impairment (e.g., protein or casts are detected) or in whom surgical treatment of BPH is being considered, specialized testing is performed. Serum creatinine may be assessed to check for evidence of chronic renal disease. If present, such patients have a higher risk of postoperative complications than patients with normal renal function, 25% versus 17%, respectively, and of worsening renal function due to radiographic contrast media, if used during imaging to assess renal function and anatomy.[52] In such high-risk patients, a renal ultrasound would be a better test for evaluation of renal anatomy.

Routine objective testing includes uroflowmetry, postvoid residual urine volume, and transrectal ultrasound of the prostate, all of which are discussed below. In addition, cystoscopy may be performed. Cystoscopy requires that an endoscope is passed transurethrally so that the urologist can visualize the urethra, bladder neck, and bladder. In patients with BPH, the classic findings are muscular changes in the bladder wall (specifically, smooth muscle hypertrophy) secondary to prolonged bladder neck obstruction and obstruction of the urethral lumen and bladder neck by an enlarged prostate. This gives the appearance that the three sidewalls of the prostatic urethra bulge out and appear to kiss each other.

American Urological Association Symptom Index Score, International Prostate Symptom Score, and Benign Prostatic Hyperplasia Impact Index

The AUA-SI score is a validated survey instrument is administered to the patient, who responds to a series of seven questions about the severity of his obstructive and irritative voiding symptoms.[52,53] For each question, the patient rates symptom severity on a scale of 1 to 5, where 0 is not at all bothersome and 5 is almost always bothersome. Thus, the lowest total score is 0 and the maximum total score is 35. Scores are interpreted according to the following ranges:

- No or mild symptoms, score of 0–7
- Moderate symptoms, score of 8–19
- Severe symptoms, score of 20–35

The AUA-SI score is administered to establish a baseline and then is repeated at regular intervals for patients with mild symptoms to determine if symptoms are worsening over time and deserve medical or surgical treatment. Similarly, once specific treatment for moderate or severe symptoms of bladder outlet obstruction is initiated, the AUA-SI score is repeated several weeks after treatment is started to determine if the treatment is effective in relieving symptoms. An effective treatment should reduce the AUA-SI score by 30–50% or decrease the score by at least three points.

The IPSS is a symptom survey instrument, which includes all seven questions in the AUA-SI score survey plus one additional question about the impact of the patient's voiding symptoms on overall quality of life. The last question is not included in the total score. Therefore, the total score ranges from 0 to 35, with 0 suggesting that the patient has no symptoms and 35 suggesting that the patient has severe symptoms.

Both the AUA-SI score and the IPSS may not correlate with the actual severity of the patient's obstruction. This is partly because some patients deny the presence of LUTS and attribute their symptoms to getting older. Furthermore, the AUA-SI score and the IPSS do not always correlate with prostate gland size, urinary flow rate, or postvoid residual urine volume. However, patients with a high AUA-SI score and a high IPSS generally show significant improvement with surgical treatment for BPH.[53]

The BPH Impact Index is a four-question tool. For the first three questions, the patient can rate voiding symptoms on a scale of 0 (not bothersome) to 3 (causes a lot of bother). The fourth question focuses on the impact of the patient's voiding symptoms on daily activities, and this is rated on a scale of 0 (no impact) to 4 (impacts my activities all of the time).

Digital Rectal Exam of the Prostate

Because of its location below the urinary bladder, the prostate is difficult to examine directly. Instead, it must be examined indirectly by having a physician insert a gloved index finger into the anus and then digitally palpating the prostate through the rectal wall. This is a simple physical examination procedure, which can be performed without any local anesthetic or bowel preparation. The prostate is assessed for its size, shape, consistency, and mobility. A normal prostate is 15–20 g in size, is heart-shaped and symmetric, has a soft consistency similar to the thenar eminence of the hand with no areas of nodularity or induration, and should be moveable when pushed with the finger. Patients with BPH have an enlarged, symmetric, rubbery, mobile gland with a smooth surface. In contrast, a patient with prostate cancer could have a variable size (normal-sized or enlarged), asymmetric gland with a nodular or indurated surface on palpation. If the cancer has locally extended to surrounding periprostatic tissue, the prostate becomes fixed in place and is no longer mobile.

The physician will estimate the size of the gland based on the degree to which the examiner's finger can reach up to the base and over the border of the prostate gland. The accuracy of the prostate size assessment by digital rectal exam is dependent on the expertise of the clinician who is conducting the exam. Because of skill variability among clinicians for this physical assessment technique, a transrectal or transabdominal ultrasound is often performed to better assess the size of an enlarged gland.[54]

An accurate prostate size assessment is useful for identifying patients at high risk for developing complications of BPH who would most benefit from treatment with 5α-reductase inhibitors. These agents are most effective in patients with prostates that are least 40 g in size, and treatment can reduce the risk for acute urinary retention, slow BPH progression, and delay the need for surgery.[55,56] In addition, the size of the prostate helps determine the best surgical approach (i.e., transurethral versus open) for large prostate glands.

Estimated prostate size does not correlate with the severity of voiding symptoms or degree of bladder neck obstruction.[52,57] This can be explained by the existence of at least two mechanisms for obstructive voiding symptoms in patients with BPH. In some patients, the obstructive voiding symptoms are due to the anatomic blockade of the urethra caused by the enlarged prostate gland. However, in other patients, obstructive voiding symptoms may be due to excessive α-adrenergic stimulation of receptors in the smooth muscle fibers of the prostate and bladder neck, which decreases the caliber of the urethral lumen. In these patients, despite the absence of a significantly enlarged prostate gland, the patient may develop significant symptoms.

Alternatively, some patients with BPH have enlargement of the median lobe of the prostate, which grows inside the bladder and produces a ball-valve obstruction of the bladder neck. In this case, the enlarged gland is not palpable on digital rectal exam but must be identified by transrectal ultrasound of the prostate or cystoscopy.

Peak Urinary Flow Rate

Peak urinary flow rate, normal range:
≥25 mL/sec, in young male
≥10–15 mL/sec, minimum, in older males

The *peak urinary flow rate* refers to the speed with which urine is emptied out of a full bladder. It is assessed as a simple outpatient procedure. The patient is instructed to drink water until his bladder is full and is then instructed to urinate into the uroflowmetry measuring device until he feels empty. The peak urinary flow rate is the maximum flow rate using the time period limited to the interval when the bladder volume was at least 150 mL.[57] The average urinary flow rate is calculated from the total volume (mL) of urine collected divided by the total time (seconds) that it took to empty his bladder.

A low peak urinary flow rate is suggestive of bladder outlet obstruction, particularly when the peak urinary flow rate is <10–12 mL/sec. In addition, a patient with a peak urinary flow rate of <10 mL/sec is more likely to benefit from surgical correction of BPH than a patient with a higher flow rate.[52] However, there is no direct correlation between voiding symptom severity and urinary flow rate. Again, this is likely due to patient's attribution of voiding difficulty to advancing age (and not due to a prostate disorder), or a patient's denial of the presence of symptoms.[52]

There is no standardized cutoff point for urinary flow rate that identifies a patient with clinically significant urinary obstruction requiring medical or surgical treatment.[58] In patients with BPH, the urinary flow rate is typically used along with the patient's AUA-SI score and the absence or presence of complications secondary to bladder neck obstruction to assess the severity of the patient's disease.[57] As mentioned, a patient may have a low urinary flow rate but may not consider his symptoms severe. In this case, the perceived severity of the patient's symptoms will impact on the ultimate choice of therapy for the patient rather than the urinary flow rate.

A limitation of uroflowmetry testing is that there is intrapatient variability of results from test to test. That is, even if repeated on the same day, the urinary flow rate may not be the same in the same patient.[52] Also, a low flow rate is not specific for BPH. Low flow rates may be due to urethral stricture, meatal stenosis, or neurogenic bladder secondary to detrusor muscle hypotonicity.[57,58] The latter occurs in patients with diabetes mellitus, peripheral neuropathy, or spinal cord injury.[58]

Postvoid Residual Urine Volume

Normal range: <50 mL

Postvoid residual urine volume refers to the amount of urine left in the bladder after a patient empties his bladder and voids a minimum volume of 120–150 mL. In a normal person, the postvoid residual urine volume should be zero, usual range 0.09–2.24 mL, but may be as high as 12 mL.[59] However, in patients with BPH, the enlarged prostate at the bladder neck causes an obstruction that makes it difficult to empty urine completely from the bladder. Chronic retention of large volumes of urine increases the risk of urinary tract infections in men with BPH and can lead to decompensation of the detrusor muscle fibers of the urinary bladder, which can result in urinary frequency, overflow incontinence, or urinary retention.

Traditionally to assess the postvoid residual urine volume, the patient is asked to empty his urinary bladder. Then a small bore urethral catheter is inserted up the urethra and into the urinary bladder to drain any residual urine. The urine is collected and the volume is measured. This method is invasive and is associated with some risk of urethral injury and pain secondary to catheter insertion. More recently, noninvasive determination of the postvoid residual urine volume with abdominal ultrasonography is commonly used.

A specific postvoid residual urine volume has not been identified as a critical value that necessitates treatment, although in clinical practice, a persistent postvoid residual urine volume of 50 mL or more is cause for concern.[52] Although a high postvoid residual urine volume correlates with decreased peak urinary flow rate, the former may not correlate with the patient's reported symptom severity.[60,61] However, effective drug or surgical treatment that improves symptoms of BPH generally reduces a high postvoid residual urine volume. As a result, clinicians generally evaluate elevated postvoid residual urine volumes in the context of the patient's medical history of recurrent urinary tract infections.

A high postvoid residual urine volume is not specific for BPH. An enlarged prostate due to prostate cancer can be associated with an increased postvoid residual urine volume. Also, a hypotonic detrusor muscle, which lacks contractile force to empty the bladder, as occurs in patients with peripheral neuropathies secondary to severe diabetes mellitus, spinal cord injury, or chronic alcoholism, can be associated with a high postvoid residual urine volume.

Transrectal Ultrasound of the Prostate

Normal prostate size: <15–20 cm^3

In this outpatient procedure, after application of a local anesthetic jelly to the rectal mucosal surface, biplanar ultrasound probes are inserted into the rectum. Ultrasound waves are bounced through the rectal wall to assess the size, shape, and echogenicity of the prostate. Transrectal ultrasound of the prostate is more accurate in estimating prostate size than digital rectal exam and helps inform the urologist of the best approach for surgical removal of an enlarged prostate gland.

Transrectal ultrasound of the prostate also is used to assess indurated or nodular areas of the prostate in a patient with an elevated PSA. A transrectal ultrasound of the prostate may reveal hyper isoechoic, hypo isoechoic, and isoechoic areas of the prostate. By so doing, different sites for prostate needle biopsy can be better identified.[60] (**Minicase 2**.)

PROSTATE CANCER

Prostate cancer is the most common cancer of American men, and the second leading cause of cancer-related death among American men. The prevalence is highest in males 50 years or older, and the median age at diagnosis is 66 years old.[62] The clinical presentation of prostate cancer is variable. In some patients, prostate cancer is slow growing and may or may not be associated with localized symptoms, such as voiding difficulty. Such patients are more likely to die from other concurrent medical illnesses and not prostate cancer. In other patients, prostate cancer spreads quickly, follows a progressive course, and produces many systemic symptoms. Such patients are more likely to die from complications of prostate cancer and its treatment. Today, due to increased screening for prostate cancer, most patients with this disease are diagnosed when their cancer is at an early stage, and the five-year survival is 100% with surgery or radiation therapy.[63]

The symptoms of prostate cancer are associated with cancer invasion of the prostate gland or tumor spread to metastatic sites. Tumor in the prostate gland generally causes hardness, nodularity, induration, asymmetry, and also may be associated with glandular enlargement, which can lead to obstructive voiding symptoms (e.g., decreased force of urinary stream, inability to completely empty the bladder, and overflow urinary incontinence, similar to BPH). Tumor spread to bone can cause bone pain and anemia; spread to the vertebral bodies can lead to spinal cord compression resulting in peripheral neuropathies, urinary or fecal incontinence, or difficulty walking; spread to the lymph nodes can cause lymphadenopathy, lower extremity peripheral edema, or ureteral obstruction; and spread to the rectum can cause rectal bleeding.[64]

When a patient has an elevated PSA or a suspicious finding on a digital rectal exam, an ultrasound-guided (or an ultrasound-guided biopsy combined with magnetic resonance imaging) prostate needle biopsy is performed. A tissue-diagnosis of prostate cancer confirms the presence of the tumor. Based on the Gleason score of the tumor specimen, PSA, digital rectal exam, transrectal ultrasound of the prostate, and a variety of other tests to check for extraprostatic spread (**Table 23-13**),

MINICASE 2

A Patient with Lower Urinary Tract Symptoms and an Enlarged Prostate

Sam B. is a 70-year-old, white male who complains of poor quality sleep. He feels tired all the time when he is awake. He falls asleep when he is driving. He says that "he can't live like this."

Cc: Sam B. has to urinate three times a night, sometimes four, and he can't get a good night's sleep. His nocturia began about four months ago. He has reduced caffeine intake and stopped drinking fluids in the evening to control his symptoms, but he has noticed minimal improvement.

HPI: Sam B. reports that over the last four years, his symptoms gradually worsened. He used to get up only once a night to urinate, but over time, the number of nighttime voidings has increased. Now he hardly falls asleep before he has the urge to urinate. He reports no urinary incontinence or blood in his urine. He also states that he has not been treated for urinary tract infections. The last visit to his urologist was one year ago, and he was told to reduce caffeine intake and withhold fluids three hours before bedtime. No medications were prescribed. At that time, the urologist told him that his problem was a natural part of getting old, that his prostate was big, and that he should not worry.

Sam B. remembers that the urologist ran some tests, but he does not remember the results.

PMH: Diabetes mellitus, type II

Medications: Glipizide

Allergies: cephalexin (skin rash, all over his trunk)

Physical exam:

ROS: Well-developed, well-nourished male with nocturia × 3

Vital signs: BP 138/85 mm Hg, HR 70 beats/min, RR 15 breaths/min, temperature 98.6 °F, weight 80 kg, height 5'10"

Genitourinary tract: Normal penis, no curvature; testes, mildly atrophic; anal sphincter tone intact and within normal limits; digital rectal exam reveals enlarged, symmetric, soft prostate, approximately 40 g, no nodules or induration; pedal pulses +3, bilaterally

Laboratory results: SMA-6 all results are within normal limits, A1c 4%, PSA (from two weeks ago) 1.8 ng/mL

Urinalysis: No bacteria, white blood cells, red blood cells, or crystals on microscopic examination

Assessment: Nocturia most likely due to an enlarged prostate; patient is not taking any medications that would exacerbate LUTS; diabetes mellitus is well controlled, has no symptoms that would contribute to LUTS

Plan: Conduct some assessments to determine the severity of the patient's lower urinary tract symptoms.

QUESTION: From the test results below, assess the severity of this patient's disease:

AUA-SI score: 21

Peak urinary flow rate: 7 mL/sec

Postvoid residual urine volume: 10 mL

DISCUSSION: The AUA-SI score indicates that the patient perceives his voiding symptoms as severe. The peak urinary flow rate is below the normal range, which indicates that he has significant obstruction to urinary outflow from the bladder. However, he seems to be able to empty urine out of the bladder, as the postvoid residual urine volume is in the normal range. The patient also reports that he has not been treated for urinary tract infections. In addition, the digital rectal exam reveals a large prostate with no signs consistent with prostate cancer. His PSA is below the age-related normal range, which is consistent with the digital rectal exam findings of an enlarged but noncancerous prostate gland. Thus, this patient has severe lower urinary tract symptoms, most likely due to benign prostatic hypertrophy.

QUESTION: Is this patient a candidate for additional treatment for benign prostatic hypertrophy?

DISCUSSION: Yes. In addition to reducing caffeine intake and withholding fluids before bedtime, the options include medication therapy to control voiding symptoms and prevent disease progression, or surgical intervention (e.g., prostatectomy, minimally invasive surgical treatment). In general, medication therapy is tried first as it is less invasive and has fewer adverse effects than surgical intervention.

TABLE 23-13. Clinical Tests Used to Stage Prostate Cancer

CHECKING FOR METASTASES IN	INITIAL CLINICAL TEST	ADDITIONAL CLINICAL TESTS IF INITIAL CLINICAL TEST IS POSITIVE
Bone	Bone scan	Bone survey (radiograph of entire boney skeleton)
Lung	Chest x-ray	Chest CT scan
Liver	Liver function tests	CT scan of the abdomen
Lymph nodes	PSA >20 ng/mL or Gleason score of 8–10; or peripheral edema on physical exam; or high volume disease on prostate needle biopsy	CT scan of the pelvis
Periprostatic tissue (e.g., seminal vesicles, fat tissue)	Digital rectal exam of the prostate, transrectal ultrasound of the prostate	

CT = computerized tomography; PSA = prostate specific antigen.

a clinical stage of disease can be determined and a risk assessment for tumor recurrence can be performed. If the patient has low volume disease on biopsy confined to the prostate, a PSA <10 ng/mL, a Gleason score ≤6, and is considered to be at low risk of tumor recurrence, no further testing is warranted.[65] On the other hand, if the patient has localized disease but is at high risk for tumor recurrence, aggressive treatment is usually indicated.

Screening for prostate cancer using PSA with or without a digital rectal exam is recommended for men with at least a ten-year life expectancy. In the past, such screening was performed annually, but it resulted in overdiagnosis of prostate cancer such that patients were subjected to unnecessary and repeated prostate needle biopsies and overaggressive surgical or radiation treatment of the slow growing, noninvasive, or indolent tumor. Not only are these measures associated with adverse effects, but they are costly to the healthcare system.[66] In addition, overdiagnosis was associated with improved life expectancy in only about 20% of patients.[66] Finally, there is no threshold PSA below which the absence of prostate cancer is guaranteed. In the Prostate Cancer Prevention Trial, men with PSAs ≤0.5 ng/mL, 0.6–1 ng/mL, 1.1–2 ng/mL, 2.1–3 ng/mL, and 3.1–4 ng/mL had a 6.6%, 10%, 17%, 23.9%, and 26.9% prevalence of histologically confirmed prostate cancer, respectively. Of these cases, 10–25% had high-grade tumors, which generally carry a worse prognosis than low-grade tumours.[67]

As a result, many professional organizations have taken a more conservative position on who should be screened, when should screening begin, and how often screening procedures should be repeated. Excellent reviews have been published recently.[68,69] Despite differing opinions among various professional organizations on prostate cancer screening, the following statements are agreed on (a sampling is shown in **Table 23-14**):

- PSA can be used alone as a prostate cancer screening tool. However, its specificity is improved when combined with a digital rectal examination, performed by an experienced examiner, and is associated with minimal adverse effects.[70]
- Lengthening the interval between repeat PSA tests from one to two or more years increases the specificity of PSA when used for prostate cancer screening and reduces the potential harms of screening. This is particularly applicable to patients with very low PSA levels (<1 ng/mL).
- PSA screening should be offered only to men with a life expectancy of at least 10–15 years.
- Patients at high risk of prostate cancer include African-American males and those with a family history of prostate cancer (father or brother with prostate cancer). These patients should be screened starting at an earlier age and more frequently than patients with average risk factors for prostate cancer.
- Patients with low-stage, low-grade prostate cancer will not require immediate treatment for prostate cancer. However, they should be managed by active surveillance with repeat PSA testing and prostate biopsies at regular intervals to check for upstaging or upgrading of the disease.[68]
- Patients vary in terms of the value they place on diagnosing and treating prostate cancer early, and this should impact the patient's and physician's decision making with regard to the timing and frequency of prostate cancer screening for an individual patient.[68]

TABLE 23-14. Comparison of Prostate Cancer Screening Recommendations by Selected Professional Organizations[68-76]

ORGANIZATION	AGE (yr) AT WHICH SCREENING SHOULD START FOR HIGH-RISK PATIENTS	AGE (yr) AT WHICH SCREENING SHOULD START FOR AVERAGE-RISK PATIENTS	AGE (yr) AT WHICH SCREENING IS NO LONGER RECOMMENDED	RECOMMENDED FREQUENCY OF PSA SCREENING
American Cancer Society	40 or 45, depending on level of risk for prostate cancer	50	If life expectancy is <10 yr	If PSA is 2.5 ng/mL or higher, repeat annually If PSA is <2.5 ng/mL, repeat every 2 yr
U.S. Preventive Services Task Force	Not recommended			
National Cancer Institute	Insufficient evidence to recommend PSA or digital rectal exam			
American Urological Association	40	55—shared decision making recommended	70 or if life expectancy is <10–15 yr	Every 2 yr for average-risk patients
American College of Physicians	40–45	50—shared decision making recommended	70 or if life is <10–15 yr	If PSA is ≥2.5 ng/dL, repeat annually
Canadian Task Force on Preventive Health Care	Not recommended			
Canadian Urological Association	40	50	75 or if life expectancy is <15 yr (unless patient had a high PSA previously)	If PSA is <1 ng/mL, repeat every 5 yr Every 2 yr for average-risk patients Men with PSA above the age-specific range, should have more regular screening

Because of PSA's limitations as a screening tool, the U.S. Preventative Services Task Force does not recommend routine prostate cancer screening using PSA. Instead, it recommends that physicians candidly discuss with each patient the option of routine screening versus not screening and give full consideration to the patient's age, willingness to live with cancer, overall health, and side effects of treatment if prostate cancer is diagnosed.[74] These considerations have been adopted by other professional organizations in their recommendations for the patient's and physician's decision making with regard to prostate cancer screening procedures. As a result of prostate cancer screening with a PSA and digital rectal exam, four common scenarios may result (**Table 23-15**).

As previously mentioned, prostate cancer screening may detect low-grade, low-stage disease in some patients who may be managed with active surveillance, which entails repeat PSA monitoring and serial prostate biopsies. No single active surveillance regimen has been accepted as a gold standard. In addition, PSA does not distinguish between indolent and invasive tumors. Thus, a focus for the development of new laboratory tests is to identify additional tools that can distinguish patients, at high risk for tumor invasiveness who need early aggressive treatment, from those who do not need additional prostate biopsies or frequent PSA testing (**Table 23-16**).[77] These tests have not been validated in large-scale clinical trials with long-term followup of patients, are much more expensive than PSA, may not be covered by insurance plans, and may not be available from all clinical laboratories.[78]

Current clinical tests to stage prostate cancer fail to identify approximately one third of patients with low- volume prostate cancer that has spread outside of the prostate gland. Thus, the search continues for improved diagnostic tools. For example, ProstaScint is a type of scan that uses indium-111 Capromab pendetide, a monoclonal antibody against prostate specific membrane antigen, to detect prostate cancer cells that may have spread to soft tissue outside of the prostate gland.[79] Initial evaluation shows that ProstaScint may be useful for detecting tumor recurrence or for identifying those patients with disease that has spread locally outside the prostate. However, its role as a tumor marker must be further defined.

TABLE 23-15. Typical Actions Taken Depending on PSA and Digital Rectal Exam Screening Results

IF THE RESULTS SHOW: PSA	DIGITAL RECTAL EXAM RESULT	NEXT STEP IN DIAGNOSIS
Normal	Normal	Have the patient return for repeat screening when appropriate
Normal	Abnormal	Have the patient undergo prostate needle biopsy
Abnormal	Normal	Repeat the PSA; if it remains elevated, have the patient undergo prostate needle biopsy
Abnormal	Abnormal	Have the patient undergo prostate needle biopsy

PSA = prostate specific antigen.

TABLE 23-16. New Laboratory Tests for Prostate Cancer Management

	LABORATORY TEST DETECTS THIS	BODY FLUID/ TISSUE USED TO RUN THE TEST
Used to assess the need for immediate treatment or initial biopsy	Genes that regulate the rate of prostate cancer cell division (Prolaris)	Prostate cancer tissue
	TMPRSS2:ERG gene fusion detection (ProstaVysion)	Prostate tissue
	4K score	Blood
	17 genes associated with androgen signaling and prostate cancer cellular proliferation (Oncotype DX)	Prostate cancer tissue
Used to assess the need for repeat biopsies in patients who have one or more negative biopsies	Overexpression of PCA3, a long noncoding RNA (Progensa)	Urine specimen collected after digital rectal exam
	DNA methylation (ConfirmMDx)	Prostate tissue

DNA = deoxyribonucleic acid; RNA = ribonucleic acid.

Prostate Specific Antigen

Non-age-related normal range: <4 ng/mL or <4 mcg/L
Age-related normal ranges:
Men, age 40–49 yr, 0–2.5 ng/mL (0–2.5 mcg/L)
Men, age 50–59 yr, 0–3.5 ng/mL (0–3.5 mcg/L)
Men, age 60–69 yr, 0–4.5 ng/mL (0–4.5 mcg/L)
Men, age 70+ yr, 0–6.5 ng/mL (0–6.5 mcg/L)

Prostate specific antigen (PSA) is a glycoprotein produced by the glandular epithelial cells that line the acini and ducts in the transition zone of the prostate gland. PSA is produced by both normal cells and prostate cancer cells. Small amounts also are produced by breast tissue, parotid glands, and periurethral glands. In normal, healthy males, 20–45 years of age, mean plasma PSA levels are undetectable or at the low end of the normal range, usually <1.14 ng/mL in Caucasians and <1.37 ng/mL in African Americans. This is because PSA is carried out of the prostate through ducts to the urethra, where it is passed out of the body in the ejaculate during coitus. PSA liquefies semen after ejaculation. However, once the prostate gland becomes cancerous, the duct system in neoplastic tissue is disrupted. As the gland grows, PSA production increases and leaks into the circulation; this results in elevated plasma PSA levels.

In the bloodstream, PSA exists in two forms: free PSA (fPSA) and complexed PSA (cPSA). Of the total PSA in plasma, 30% is fPSA, and 70% of the total PSA is complexed to α1-antichymotrypsin (ACT) and α2-macroglobulin (A2M).[80] FSA is renally excreted, while cPSA is hepatically catabolized. A PSA level measures both fPSA and PSA complexed to ACT.

In the bloodstream, fPSA exists in several forms. ProPSA is the inactive precursor of PSA and is associated with prostate cancer.[81] It may be modified or clipped to produce two different inactive forms; however, most of it is converted to active PSA. Active PSA can be converted to inactive PSA or benign PSA (BPSA), which is produced by BPH tissue, as opposed to normal prostate tissue. High levels of BPSA are associated with high-volume BPH and obstructive voiding symptoms.[81] Preliminary studies are being conducted to evaluate the diagnostic usefulness of measuring ProPSA, clipped forms of ProPSA, and BPSA. Currently, these forms of fPSA are largely used as research tools. Whether these will replace PSA as a tumor marker is not known at this time. The plasma half-life of PSA is two to three days. Because of daily intrapatient variation in PSA measurements, it is recommended to confirm an increased PSA value by repeating it. [80]

PSA serum levels are affected by several patient factors.[82,83] Decreased PSA levels are associated with obesity. It has been postulated that obese patients have larger circulating plasma volumes, which dilute PSA concentrations in the bloodstream.[84] Decreased PSA also is seen in hypogonadism because hypogonadism results in shrinkage of the prostate gland—the major site of PSA production. Increased PSA levels are observed in elderly patients because BPH occurs with a high prevalence, and the increased prostate volume results in an increased volume of glandular epithelial tissue—the site of PSA production. Also, increased PSA normal range levels are reported in African-American patients <60 years of age.[85] The reason for this is unknown.

As a tumor marker, PSA has several uses: (1) as a diagnostic screening test for prostate cancer; (2) to determine the spread of the disease; and (3) to assess the patient's response to treatment. As a diagnostic screening test, PSA has high sensitivity (70–80%) but low specificity (50%) for prostate cancer when used alone. The positive predictive value of PSA to diagnose prostate cancer is directly related to the PSA value such that the higher the PSA value, the higher the positive predictive value. In the range of 2.5–4 ng/mL, PSA has a positive predictive value of 18%. In the range of 4–10 ng/mL, PSA has a positive predictive value of 20–25%. Above 10 ng/mL, PSA has a positive predictive value of 42–64%.[86] PSA is commonly used in combination with a digital rectal examination of the prostate for prostate cancer screening because the combination has better sensitivity and specificity that either test alone. When used in combination with a digital rectal exam, the sensitivity of PSA increases to 85–90%, and the positive predictive value for a PSA cutoff of 4 ng/mL increases from 32% to 49%.[71]

Table 23-17 shows a common interpretation of prostate cancer screening test results for a PSA normal range of <4 ng/mL. Although the PSA level increases with the size of the tumor, there is a poor correlation between the PSA level and the actual size of the prostate tumor. However, a semiquantitative relationship exists between the PSA level and the degree of prostate cancer spread such that a PSA level <10 ng/mL suggests that the tumor is confined to the prostate; a PSA level of >20 ng/mL suggests the possibility of extracapsular spread; and a PSA level of ≥80 ng/mL suggests advanced disease.[85,89]

When used to assess the patient's response to localized treatment for prostate cancer, an elevated PSA prior to treatment should be reduced to the normal range, or at least exhibit a twofold reduction in PSA level, with effective treatment. In addition, pretreatment PSA is used along with the Gleason score of prostate tissue and the clinical stage of disease to predict the patient's posttreatment risk of disease recurrence.[87,88] This information is then used to guide treatment selection for individual patients.[87] Although multiple risk-stratification schemes have been devised, no one system is considered to be the standard.[89]

As previously mentioned, when using a cutoff value of 4 ng/mL, the PSA is 70–80% sensitive in screening for prostate cancer but has low specificity. Also, some high-grade prostate tumors produce less PSA than other prostate cancer tumors. Thus, this cutoff value for PSA potentially misses up to 27% of patients with prostate cancer confined to the prostate gland, which is curable.[80] Many noncancerous conditions can increase PSA (**Table 23-18**), which could trigger a clinical decision for an unnecessary prostate biopsy. For example, almost 30% of men with BPH have PSA values of 4 ng/mL or higher.

Medications may alter PSA levels. Of importance, the 5α-reductase inhibitors (e.g., finasteride (Proscar) and dutasteride (Avodart), generally produce an average 50% reduction in PSA after six months of continuous treatment. This has been

TABLE 23-17. Common Interpretation of Increased PSA Laboratory Values

0–3.9 ng/mL	Normal range
4–9 ng/mL	A biopsy is recommended (the probability of detecting prostate cancer is 25–30%)
≥10 ng/mL	A biopsy is recommended (the probability of prostate cancer is at least 50%)

PSA = prostate specific antigen.

TABLE 23-18. Diseases, Procedures, and Medications That Increase or Decrease (Total) PSA[75,80,90,91]

INCREASE PSA	DECREASE PSA
BPH	Obesity
Prostatitis	Hypogonadism
Prostate trauma (e.g., massage, biopsy)	Medications: α-inhibitors (e.g., finasteride, dutasteride), HMG-CoA reductase inhibitors (e.g., statins), aspirin, thiazide diuretics
	Herbals: saw palmetto
Prostate surgery or procedures[a]	
Acute urinary retention	
Ejaculation	
Exercising on a bicycle for 30 min	
Medications: parenteral testosterone supplements	
PSA bounce after radiation treatment for prostate cancer	

BPH = benign prostatic hyperplasia; HMG-CoA = 3-hydroxy-3-methylglutaryl-coenzyme A; PSA = prostate specific antigen.

[a] Procedures that have minimal effect on (total) PSA: digital rectal exam, transrectal ultrasound of the prostate, cystoscopy, and urethral catheterization.

reported with usual daily doses of both drugs (5 mg finasteride daily and 0.5 mg dutasteride daily) for treatment of BPH and also with 1 mg finasteride (Propecia) daily used for androgenetic alopecia.[92-94] To preserve the usefulness of PSA as a tumor marker in patients who are taking 5α-reductase inhibitors, it is essential to obtain a pretreatment PSA as a baseline. When PSA levels are repeated after at least six months of treatment, it is recommended to double the measured PSA level before interpreting it. If a patient has a PSA level that is significantly higher than baseline after six months of treatment, it is recommended that the patient be evaluated for causes of the abnormal PSA level, including prostate cancer. If the patient has not experienced a 50% decrease in measured PSA level after six months of treatment, it is recommended that the patient be questioned as to his adherence with the prescribed regimen.

Another interesting aspect of the effect of finasteride on PSA levels is that when finasteride was used to prevent prostate cancer, it appeared to increase the sensitivity of PSA as a screening test for prostate cancer and to improve the ability of the prostate needle biopsy to detect prostate cancer.[92,95] To minimize the impact of noncancerous conditions on PSA (Table 23-18), it is recommended to allow an adequate interval after the condition has resolved before measuring PSA. Consideration of PSA's plasma half-life of two to three days along with the time it takes the condition to resolve affects the time interval. For example, following transurethral prostatectomy, it is recommended to wait six weeks before obtaining a PSA, whereas, following ejaculation, it is recommended to wait only two days. Prostatitis produces sustained elevations in PSA until the infection or inflammation is resolved; therefore, it is recommended that PSA testing be held for up to eight weeks after symptom resolution. Also, in a patient with PSA levels in the gray zone of 4–10 ng/mL who has a normal digital rectal exam and no evidence of infection on urinalysis, a short three-week treatment course of antibiotics (to treat a presumptive prostate infection) has been used before repeating the PSA. In some cases, the PSA returns to the normal range. This strategy has been used to avoid unnecessary biopsy of the patient; however, it is considered a controversial measure at this time.[96]

An increase in PSA can occur for up to 14 weeks after docetaxel treatment of castration-resistant prostate cancer or 17–18 months after radiation therapy for prostate cancer.[97,98] This phenomenon is known as *PSA bounce* or *PSA flare*. Such increases in PSA should not be interpreted as biochemical evidence of disease relapse or should not be used to decide additional treatment for the patient. Instead, in the face of metastatic prostate cancer, other objective signs of disease progression should be used to make treatment-related decisions. A 20% biological variation in measured PSA levels has been documented when the PSA ranges from 0.1–20 ng/mL. For this reason, it is common practice to repeat a single elevated PSA and not to take action based on a single elevated value.[93]

Various alternative strategies have been employed to improve the usefulness of PSA as a tumor marker for prostate cancer screening including the following:

1. Consider the normal value of total PSA to be <2.5 ng/dL, particularly in men<60 years of age. Thus, patients with a total PSA of 2.5 ng/dL or greater would undergo a prostate needle biopsy. This should avoid missing that subgroup of patients with organ confined prostate cancer who have PSA values in the range of 2.5–4 ng/dL.[99,100] However, lowering the normal value of PSA also is likely to increase the number of biopsies that are negative.[101]
2. Consider age-related normal value ranges.[102] For the current cutoff value of 4 ng/mL, the specificity of PSA decreases as men age.[70] This is because PSA normally increases as men age and develop BPH. Thus, to minimize the risk of interpreting an increased PSA due to prostate cancer, age-related normal value ranges (which have been further delineated for Asians and African Americans) listed below are often provided by clinical laboratories (**Table 23-19**).[103,104] An advantage of age-related normal value ranges is that they increase the likelihood of disease detection in young men. However, a disadvantage is that they delay biopsies in older men, which can delay the diagnosis of prostate cancer.[80,104]

The total PSA range of 4–10 ng/mL is considered to be a gray-zone range because the increase in PSA in many cases is due to BPH and not prostate cancer. Thus, to improve the usefulness of total PSA in the range of 4–10 ng/mL as a screening test or to assess prognosis of patients, the following strategies have been recommended by some investigators:

- **PSA density (PSAD).** The PSAD is thought to be increased in patients with prostate cancer as compared to patients with BPH. The PSAD is calculated by dividing the total PSA by the prostate volume as determined by transrectal ultrasound of the prostate (TRUS). A normal PSAD is <0.15 ng/mL/cm^3. If the PSAD is 0.15 ng/mL/cm^3 or more, it suggests that the patient's increased PSA is due to prostate cancer, and this patient should undergo additional diagnostic testing. However, this cutoff value has only 50% sensitivity, and it misses

TABLE 23-19. Age-Specific Median and Normal Value Ranges for PSA in Adult Males of Various Races[103]

PATIENT AGE (years)	OVERALL MEDIAN (ng/mL)	CAUCASIANS (ng/mL)	ASIANS (ng/mL)	AFRICAN AMERICANS (ng/mL)
40–49	0.7	0–2.5	0–2.0	0–2.0
50–59	0.9	0–3.5	0–3.0	0–4.0
60–69	1.3	0–4.5	0–4.0	0–4.5
>70	1.7	0–6.5	0–5.0	0–5.5

PSA = prostate specific antigen.

many patients with prostate cancer.[104,105] In addition, to derive PSAD, a TRUS must be performed. This adds an extra cost and is usually uncomfortable for the patient. Finally, a TRUS measurement of prostate volume is difficult to reproduce in the same patient.[80]

- **PSA velocity.** The PSA velocity refers to the rate of increase in PSA values over time and is based on the concept that a faster rate of rise is suggestive of the presence of prostate cancer. To determine PSA velocity, the patient must have at least three PSA tests performed, each one is at least one year apart, or, alternatively, the patient must have three PSA tests performed over a 1.5-year period.[105] If the PSA velocity is >0.75 ng/mL/yr, this suggests that the patient has prostate cancer and should undergo additional diagnostic testing. A PSA velocity that is >0.75 ng/mL/yr has a sensitivity of 90–100% and a specificity of 95% as a screening test for prostate cancer, which is much better than total PSA. In men <60 years old, whose lifespans are potentially more severely impacted by aggressive prostate cancer, it is suggested that a PSA velocity >0.4 ng/mL/yr be used as a threshold value.[106] PSA velocity is affected by the intrapatient variation of PSA values. That is, a PSA value may fluctuate 10–25% from day-to-day in the same patient. For this reason, it may be difficult to derive a consistent PSA velocity value for a patient. Thus, some recommend that the trend of an increase in PSA values over a 1.5-year period should be considered as suggestive of prostate cancer in place of the 0.75-ng/mL/yr cutoff.[80] However, the long period of time needed to collect enough PSA measurements to determine PSA velocity is a significant disadvantage of using this strategy. It also should be noted that a recent analysis of over 5500 men in the Prostate Cancer Prevention Trial showed no advantage of PSA velocity over PSA in clinical practice.[107,108]

  A related alternative strategy is to evaluate PSA doubling time or the length of time it takes for the PSA level to double. The preoperative PSA doubling time has been used to predict cancer recurrence after radical prostatectomy. A preliminary study suggests that a PSA doubling time of less than three months indicates that the patient probably has tumor recurrence and is at high risk of prostate-cancer related death.[109] A disadvantage to using PSA doubling time is that there is no accepted standard for the minimum number of PSA values to use or the time interval between PSA values.

- **Percentage of fPSA (% fPSA).** Prostate cancer is associated with a decreased fraction of fPSA and an increased fraction of cPSA in the plasma. The percentage of cPSA is higher than normal in patients with prostate cancer because cPSA appears to resist proteolytic processing.

  Thus, if the percentage of fPSA is <25% of the total PSA and depending on the actual percentage of fPSA, the patient has up to a 56% probability of having prostate cancer (**Table 23-20**).[110,111] The use of fPSA to screen for prostate cancer when the total PSA is <4 ng/mL has not been well studied, but preliminary evaluation suggests that the percentage of fPSA may be a good screen for prostate cancer.[80] One study showed that in the (total) PSA range of 2.5–10 ng/mL, the fPSA cutoff of 25% had >90% sensitivity for screening for organ-confined prostate cancer.[111]

  Assessing the percentage of fPSA helps the clinician determine if the elevated PSA is due to prostate cancer or BPH. Thus, prostate needle biopsy would be performed on those patients with a fPSA of <10%; and patients with a fPSA of 10–25% would be advised to have a prostate needle biopsy. fPSA levels also may identify patients with aggressive prostate cancer. In one study, men 50–58 years old had a 2.4-fold increased risk of aggressive prostate cancer when the fPSA percentages were <20% when compared to men in the same age group with fPSA percentages >20%.[112] fSA is renally excreted; therefore, in patients with renal failure, the fPSA level will be increased, and the percentage of fPSA will increase.[113] fPSA increases after digital rectal exam of the prostate, prostate needle biopsy, and after ejaculation. 5α-reductase inhibitors also decrease fPSA and cPSA but do not affect the ratio of the two; therefore, fPSA percentages are not affected by these medications. fPSA blood specimens are subject to degradation if stored for long periods of time at ambient temperature. It is recommended that specimens for fPSA be stored at −70 °C or assayed within three hours of specimen collection.

- **cPSA.** As previously mentioned, prostate cancer is associated with an increased fraction of cPSA. With this assay, the concentration of PSA complexed to ACT and A2M is measured. Using the PSA normal value of 4 ng/mL, the cPSA normal value is 3.1 ng/mL. Although cPSA assays appear to be comparable in sensitivity to but have higher specificity than total PSA assays, cPSA assays have not replaced total PSA assays.[114]

  A radioimmunoassay is commonly used to measure total and fPSA levels. Assays are quick to perform and commonly available. Newer commercially available assay kits allow for measurement of PSA concentrations that are <0.1 ng/mL. Several different immunoassays are available, and results are not interchangeable among them. Therefore, it is recommended that the same

TABLE 23-20. Estimated Probability of Prostate Cancer Depending on the Percentage of Free PSA[110,111]

% FREE PSA RANGE	% PROBABILITY OF PROSTATE CANCER
0–10	56
10–15	28
15–20	20
20–25	16
>25	8

PSA = prostate specific antigen.

assay methodology be used when interpreting serial PSA results in an individual patient.[115] (**Minicase 3.**)

Prostate Needle Biopsy and Gleason Scoring of the Biopsy Specimen

A needle biopsy of the prostate is used to establish a tissue diagnosis of prostate cancer. It may be performed transrectally in one of three ways: digitally guided, guided by TRUS, or guided by real-time ultrasound fused to magnetic resonance imaging.[116]

As a digitally guided procedure, a biopsy needle is passed over the index finger of the urologist into the rectum and is directed to the site in the prostate where induration or a nodule was palpated. The needle is inserted through the rectal mucosa into the prostate to obtain a core of suspicious tissue. In addition, the urologist obtains four to six other biopsy specimens from the base, lateral mid-portion, and apex of the prostate gland. The false-negative rate with this technique is 20–25%.[117] Alternatively, a biopsy gun is used along with transrectal ultrasound for guidance. Local anesthesia is required. To reduce the false-negative rate, the number of random biopsy specimens is increased from 6 or 8–20.[118,119] With the increase in tissue sampling, the false-negative rate is only 4%.[120]

All biopsy specimens are sent to the pathologist for examination. If prostate cancer is detected microscopically, the sample is graded histologically. The *Gleason scoring* system is used to grade the pattern of glandular differentiation of the prostate tumor. Two grades are assigned: one for the dominant pattern of glandular differentiation and a second for the less prevalent pattern. Uniform, round well-formed cells would be graded as 1 or 2, whereas solid sheets of tumor cells without gland formation would receive a grade of 5. Transition between these two extremes would be graded as 3 or 4. Two grades are assigned if two patterns of infiltration are identified; or, if only one pattern of infiltration is evident, the same number is assigned twice. The two numbers are then added to give the Gleason score, which can range from 2–10. The Gleason score correlates with progression of the tumor and the patient's prognosis; the higher the score the worse the prognosis. A single tissue specimen score of 4 or more, or a total score of 7 or higher suggests that the patient is at intermediate or high risk of developing metastatic disease.[124] Along with other parameters, the Gleason score has been incorporated into various formulae to predict the prognosis of patients.[121] (Minicase 3.)

Sometimes that biopsy specimens yield suspicious findings that are not clearly identified as prostate cancer. The pathology report will read prostatic intraepithelial neoplasia or atypical small acinar proliferation in this case. Prostate needle biopsy is an invasive procedure. It can be painful and result in minor bleeding and infection. Severe adverse effects requiring hospitalization can occur in 1% of patients.

PCA3

Unlike PSA, which is secreted by both normal prostate and prostate cancer cells, *PCA3* (also known as *DD3 prostate-specific gene* or *differential display code 3*) is a noncoding RNA marker that is overexpressed in 95% of prostate cancers. Also, unlike PSA, PCA3 does not increase with patient age or prostate volume.[122]

When used along with PSA and other objective patient data, a PCA3 test is used to help decide whether a repeat prostate biopsy is not necessary (because it would likely be negative) in a patient, who has already had one or more negative prostate biopsies for prostate cancer and has a PSA of 2.5-4 ng/mL. The cutoff PCA3 score is 25. If the PCA3 score is <25, it is considered a negative test, which is interpreted that it is unlikely for the patient to have a positive biopsy if a biopsy were to be repeated. If the PCA3 score is 25 or higher, it is considered a positive test, which is interpreted that it is likely that the patient will have a positive biopsy if a biopsy is performed. Using this cutoff score in patient sample, the PCA3 test exhibits a 77.5% sensitivity and specificity of 57.1%.[123]

PCA3 should be reserved for patients who are at least 50 years of age and have PSA values of <4 ng/mL. The use of this test may decrease the number of unnecessary biopsies in patients at low risk of prostate cancer recurrence.[124] The PCA3 test is conducted on a urine specimen that is collected immediately after a digital rectal exam of the prostate, in which each lobe of the prostate is stroked three times. During this procedure, prostate cells are shed into the urine and then analyzed in the urine specimen. The PCA3 assay is comprised of two nucleic acid amplification steps. The PCA score is based on the ratio of PCA3 RNA copies to the PSA RNA copies.

The PCA3 assay should not be used in patients when the most recent prostate biopsy shows atypical small acinar proliferation, when a biopsy being considered is less than three months old or more than seven years old after the last biopsy, or in men who have never had a prostate needle biopsy.[125] PCA3 assay results may be altered by radiation therapy to the prostate, prostatectomy, or 5α-reductase inhibitor use.

4K Score

The *4K score* is based on measurement of four kallikrein markers in the blood: total PSA, fPSA, intact PSA, and hK2, an enzyme known as human kallikrein which is thought to promote cancer growth. The 4K score is meant to complement total PSA, particularly in patients who have PSAs in the range of 2–10 ng/mL. It can help distinguish patients who do not have cancer or have an indolent cancer from those with high-risk/high-grade cancer that should be biopsied and treated.[126,127]

In a limited number of studies, the 4K score reduced the number of biopsies by 30–58% in patients with PSAs that were 2 ng/mL or higher and identified almost 60% of men age 50 years or older with high grade prostate cancer.[127] However, additional examination of this assay is needed to identify the 4K score cutoff as well as the sensitivity and specificity of the assay. This assay is not yet commercially available.

TMPRSS2:ERG Gene Fusion

TMPRSS2:ERG gene fusion is a marker for prostate cancer that can be identified in biopsied prostate tissue and urine. It is present in 50% of prostate cancer. The assay for this marker exhibits 99% specificity for prostate cancer. When combined with

MINICASE 3

Interpreting PSA in a Patient with Prostate Cancer

Robert R. is a 60-year-old, white male with newly diagnosed adenocarcinoma of the prostate cancer. He is undergoing cancer staging and risk assessment to determine the next step in his management.

Cc: Robert R. is anxious and concerned about his diagnosis. He has a 10-year-old daughter and an 8-year-old son and expresses fear that he "will not be around" as they grow older.

HPI: On a routine annual physical exam, Robert R. has an abnormal digital rectal exam. The prostate is enlarged and asymmetric. A 1-cm indurated area is palpated and subsequently biopsied, which shows adenocarcinoma, Gleason grade 8 in 60% of the biopsy specimens. Two PSA tests, conducted one week apart before the prostate needle biopsy, reveal 25 g/mL and 28 ng/mL. Liver function tests, BUN, and serum creatinine are all normal.

PMH: Hypertension; leg cramps occasionally at night

Medications: Lisinopril 20 mg orally once a day; aspirin 325 mg orally once a day

Allergies: None

Vital signs: BP 135/80 mg Hg, HR 80 beats/min, RR 15 breaths/min, weight 190 lb

QUESTION: What does the patient's PSA suggest about the stage of his disease? What additional tests should be performed to confirm the stage of prostate cancer in him?

DISCUSSION: Because the patient's PSA is >20 ng/mL and the prostate cancer is a Gleason grade 8 in a majority of the specimen, he is at high risk of tumor metastasis, and his cancer has likely spread outside of the prostate. To determine the sites of tumor spread, additional testing should include a bone scan, magnetic resonance imaging of the prostate, computerized tomography (CT) scan of the pelvis, and chest radiograph.

QUESTION: Let us assume that the bone scan shows no boney metastatic sites, the chest radiograph shows no lung metastases, and the CT scan of the pelvis shows no lymphatic invasion. However, the digital rectal exam PSA and the magnetic resonance imaging of the prostate suggest direct extension of the prostate through the prostatic capsule to the periprostatic tissues, including the seminal vesicles. The patient has clinical stage T3 prostate cancer. How would this information be used in a risk assessment?

DISCUSSION: Assessment of the risk of tumor recurrence is used to guide treatment selection. Although no single risk assessment tool has been accepted as a standard, most risk assessments are similar in that they are based on the prostate cancer disease stage, PSA, and Gleason score of the tumor. One commonly used risk assessment is depicted below.[65]

As an example, for the low-risk category, a patient with stage T1C or T2A disease, a PSA <10 ng/mL or a Gleason score <6, could be offered active surveillance if his life expectancy is <10 years, or radiation therapy or radical prostatectomy if his life expectancy is 10 years or more (**Table 23-21**). The selection of a particular treatment should be individualized based on his life expectancy, his preference, and his ability to deal with potential adverse effects or complications of various treatment options. Active surveillance has no adverse effects and entails PSA monitoring every six months and an annual prostate needle biopsy. Such close monitoring will allow the physician to detect early tumor progression to a higher stage or higher grade. Radiation therapy can be delivered as external beam or internally with seed implants into the prostate. As an adjuvant to external beam radiation therapy, androgen deprivation therapy with a combination of an LHRH agonist and an antiandrogen has been shown to be more effective in prolonging tumor-free survival over external beam radiation therapy alone for stage T2 and T3 prostate cancer.[132,133] A radical prostatectomy, which includes removal of the prostate, prostatic urethra, pelvic lymph nodes, and periprostatic tissues is an effective treatment modality for localized prostate cancer and lowers the likelihood of disease recurrence and decreases cancer-related mortality. However, perioperative complications include bleeding and infection. Late complications include urinary incontinence and erectile dysfunction.

Based on this patient's disease stage, Gleason score, and PSA, he is considered at high risk of tumor recurrence and the best treatment option would be external radiation with adjuvant androgen deprivation therapy.

TABLE 23-21. Example Stratification of Risk for Disease Progression Based on Disease Stage, PSA, and Gleason Score

RISK CATEGORY FOR DISEASE PROGRESSION	STAGE OF PROSTATE CANCER[a]	PSA (ng/mL)	GLEASON SCORE	TREATMENT OPTIONS
Low	T1C or T2A	<10	6 or less	Active surveillance if life expectancy <10 yr; surgery if life expectancy ≥10 yr
Intermediate	T2B	10–20	7	If life expectancy ≥10 yr, radiation therapy with or without androgen deprivation therapy or surgery
High	T2C or T3A or T3B or T3C	>20	8–10	External radiation with androgen deprivation therapy; or surgery in selected cases

PSA = prostate specific antigen.

[a] Stage T1 and T2 are localized to the prostate. Stage T3 refers to cancer that has directly extended to periprostatic tissue. Stage T4 is metastatic to lymph nodes, bone, or soft tissues distant from the prostate. The alphabetic letter refers to the volume of the prostate cancer tissue. A refers to one focus; B refers to two foci; and C refers to multiple foci of tumor.

PCA3, TMPRSS2:ERG gene fusion testing has high sensitivity and high specificity for detecting prostate cancer and is better at predicting the clinical stage of the disease in patients when compared to PSA alone.[128,129] This assay is not altered by patient age, prostate tumor size, or the Gleason grade of the tumor.[130] This assay may be ordered from the University of Michigan and Gen-Probe.[129]

DNA Methylation Assay

DNA methylation is a common chemical modification that can result in altered gene expression, which leads to malignant cell transformation, tumor invasion, and angiogenesis.[131] The commercially available Confirm MDX assay quantitatively assesses DNA methylation in three separate genes in prostate tissue that histologically looks normal and is near a prostate tumor. An abnormality in any one of these genes is considered a positive test.

This test is for patients who have had a negative prostate needle biopsy, but the PSA level is equivocal. In these patients, a negative DNA methylation test has a high negative predictive value; that is, if the test is negative, then there is up to a 90% likelihood that there will be no cancer on subsequent biopsy. Thus, this test could avoid unnecessary biopsies in patients who do not have prostate cancer. Prostate infections and inflammation may interfere with assay results. In addition, the DNA methylation assay does not distinguish between high-grade versus low-grade prostate cancer.[131]

PROSTATITIS

Prostatitis is the most common genitourinary tract disorder among men <50 years of age. The lifetime prevalence is 15–16%.[134,135] Prostatitis is an inflammatory condition of the prostate gland due to infection or a noninfectious cause. Risk factors for prostatitis include BPH, lower urinary tract Infection, sexually transmitted diseases, and stress.[136] The National Institutes of Health (NIH) has stratified patients with prostatitis into four unique categories (**Table 23-22**). Of these, only the first two categories—acute and chronic prostatitis—have infection as the etiology and are generally responsive to antibiotic treatment. For the other two categories—chronic pelvic pain syndrome and asymptomatic inflammatory prostatitis—the etiology is unclear, which accounts for the low response rates to existing treatments. Chronic pelvic pain syndrome can be inflammatory or noninflammatory (i.e., inflammation is evidenced by the presence of WBCs, the expressed prostate

TABLE 23-22. National Institutes of Health Categories of Types of Prostatitis[138]

CATEGORY	SYMPTOMS/SIGNS	% OF PROSTATITIS CASES	INFECTIOUS ETIOLOGY	RESULTS OF 4-GLASS SPECIMEN COLLECTION METHOD
Acute bacterial prostatitis	Acute onset of urinary frequency, urgency, dysuria; perineal or suprapubic pain; and urinary retention; may be associated with fever, chills, rigors nausea, vomiting, malaise, myalgia, lower abdominal or suprapubic discomfort perineal or rectal pain; swollen, warm, tense, boggy, tender prostate on palpation; urinalysis shows significant WBCs and bacteria; blood cultures are often positive, urine culture is positive	2–5	Yes	VB1 and VB2 are positive for infection
Chronic bacterial prostatitis	History of recurrent urinary tract infection; episodes of perineal, penile, suprapubic pain; frequency, urgency, and dysuria, which are separated by asymptomatic periods; symptoms are present for a minimum of 3 mo duration; prostate may be normal on digital rectal exam, may be mildly tender and boggy, or focally indurated with crepitation; urinalysis shows significant WBCs; prostatic fluid is purulent	2–5, up to 15% in some studies	Yes	EPS and VB3 are positive for infection There is a 10-fold or more increase in bacteria in VB3 when compared to VB1 or VB2 WBCs are present in EPS and VB3
Chronic pelvic pain syndrome which can be classified as inflammatory or noninflammatory	Waxing and waning dull aching perineal, suprapubic, scrotal or penile pain; pain on ejaculation, may be associated with frequency, urgency, and dysuria; minimum of 3 mo duration; digital rectal exam is unremarkable; this is stratified into inflammatory and noninflammatory disease	90–95	No	All specimens are negative for infection; patients with inflammatory disease have WBCs in EPS,VB3, and semen; patients with noninflammatory disease have no WBCs or bacteria in EPS,VB3, or semen
Asymptomatic inflammatory prostatitis	No symptoms; this is incidentally diagnosed on histological review of a prostate tissue biopsy specimen; digital rectal exam is unremarkable	Unknown	No	WBCs in EPS, VB3, or semen

EPS = expressed prostatic secretion; VB1 = first 10 mL of urine voided; VB2 = midstream urine collection; VB3 = first 5–10 mL of urine after prostate massage; WBCs = white blood cells.

secretion [EPS], semen, or in tissue removed from the prostate during prostatectomy or biopsy).[137,138]

Differentiation among the types of prostatitis is largely determined by clinical presentation of the patient, digital rectal exam of the prostate, and the laboratory analysis of EPS (Table 23-22). Digital exam of the inflamed prostate is described as boggy or having a softer consistency than usual. Because of the concern that prostate massage could expel bacteria from the prostate into the bloodstream, prostate massage is not performed in patients with suspected acute prostatitis. Instead, symptoms and blood and urine cultures are used to diagnose the disease.

EPS is key for diagnosing chronic bacterial prostatitis, chronic pelvic pain syndrome, and asymptomatic inflammatory prostatitis, and it is collected after a prostate massage in which the prostate is stroked from side to side and then from top to bottom during a digital rectal exam for two to three minutes (the resulting fluid is collected as it drips out of the urethral meatus).[138,139]

To assess symptoms and their severity, the NIH has devised a Chronic Prostatitis Symptom Index, which is a self-assessment tool comprised of nine questions that focus on the quality and intensity of the patient's pain, urinary voiding symptoms, and the impact of the symptoms on the patient's quality of life. The total score ranges from 0–43; the higher the score the worse the symptoms. A subscore for pain and urinary symptoms also can be calculated. The subscore range is 08–31 with 0–9 indicating mild symptoms, 10–18 indicating moderate symptoms, and 19–31 indicating severe symptoms. This tool is used for a baseline assessment and then repeated at regular intervals during the course of the patient's care. This symptom survey is considered a reliable and valid instrument and is commonly used in practice to assess a patient's response to treatment or disease progression.[134,137]

Chronic pelvic pain syndrome is associated with a plethora of symptoms, and treatment is directed at specific symptoms. As a tool for selecting specific treatments for symptomatic relief, UPOINT is a clinical classification system for a patient's symptoms. Six symptom domains are used: urinary, psychosocial, organ specific, infection, neurologic/systemic, and tenderness. Based on the symptom classification of a particular patient, specific treatments are indicated.[140] The UPOINT tool is available online at www.upointmd.com.[141]

4-Glass Versus 2-Glass Method of Specimen Collection

The classic method for collecting a specimen to diagnose chronic prostatitis is the 4-glass specimen collection method.[137,142] The specimens include the following:

- **Glass 1 or voided bladder (VB1) specimen (first 10 mL of urine)**—This represents the urethral specimen.
- **Glass 2 or VB2 specimen (a midstream urine collection)**—This represents the bladder specimen.
- **Glass 3 or EPS**—After a one to three minutes of prostate massage, EPS will drip out of the urethra over the next few minutes. This represents the prostate specimen.
- **Glass 4 or VB3 (first 5–10 mL of urine after the prostate massage)**—This sample will include any residual EPS in the urethra. This represents the prostate specimen as well.

Although the 4-glass specimen collection method has been considered the standard for diagnosis, it should be noted that the method has not been validated for accuracy.[143] The diagnosis of chronic bacterial prostatitis is made when the bacterial culture in EPS or urine specimen after the prostate massage (VB3) has a 10-fold greater bacterial count as compared to the urethral (VB1) and bladder specimens (VB2).

Because of the complexity and time-consuming nature of the 4-glass specimen collection method, many clinicians use only a 2-glass method (which also is known as the *Nickel premassage and postmassage test*), collecting a VB2 and VB3 specimen premassage and postmassage, respectively. The 2-glass method produces results that are comparable to the 4-glass method, and the former has a sensitivity and specificity of at least 90% and is 96–98% as accurate as the 4-glass method.[134,144] A positive test is indicated by a 10-fold greater bacterial count in the VB3 specimen as compared to the VB2 specimen.

In addition to sending all specimens for bacterial culture, EPS and VB3 are checked for the presence of WBCs. A drop of EPS is applied to a glass side, a cover slip is placed on top, and the specimen is examined under high power on the microscope. VB3 specimens are typically centrifuged for five minutes first, and the sediment is examined in a similar fashion. The presence of >5–10 WBCs per high power field is considered significant for inflammation. Twenty or more WBCs per high power field or a VB3 with a WBC count of 1000/µL or more is diagnostic of chronic bacterial prostatitis.[137,144] Finally, it should be noted that when chronic prostatitis is strongly suspected, but EPS cultures are negative, semen specimens have been used as a substitute for EPS. However, semen cultures are only positive in approximately 50% of men with chronic bacterial prostatitis.[145,146]

SUMMARY

This chapter reviews common clinical tests and laboratory tests used for diagnosing and monitoring treatment for common urologic disorders in elderly males including late-onset hypogonadism, erectile dysfunction, BPH, prostate cancer, and prostatitis. Many of these disorders are managed with tests other than laboratory tests.

LEARNING POINTS

1. ***What is the differentiation between serum total testosterone levels and free testosterone levels?***

   ANSWER: Testosterone circulates in the bloodstream in several forms: free testosterone and testosterone bound to proteins, specifically SHBG and albumin. Most protein-bound testosterone is bound to SHBG and only a small portion is bound to albumin and corticosteroid-binding globulin. The free testosterone fraction is physiologically active. Typically, when a clinician orders a testosterone serum level, the level reflects the total testosterone concentration in the bloodstream, which includes free and protein-bound testosterone. A free testosterone serum level reflects only the unbound portion of testosterone in the bloodstream. A free testosterone level may be measured or calculated. Free testosterone serum levels may be indicated in patients in whom the concentration of SHBG is decreased or increased. In such patients, a free testosterone serum level will be a better indicator of the concentration of physiologically active testosterone. Increased SHBG is associated with cirrhosis, hyperthyroidism, old age, and drug treatment with estrogens or anticonvulsants. Decreased SHBG is associated with hypothyroidism, obesity, and drug treatment with excessive doses of testosterone supplements.

2. ***Why does the AUA-SI score not correlate with the findings on digital rectal exam or peak urinary flow rate in a patient with BPH?***

   ANSWER: The AUA-SI score is derived from a patient's self-reporting and self-assessment of the obstructive and irritative voiding symptoms due to BPH. Thus, it is a subjective assessment. It is well known that many elderly patients with BPH may deny the presence of bothersome voiding symptoms and may attribute their symptoms to their advancing age. Thus, some patients think that their problems are a normal part of the aging process and should not be treated. Other patients will make lifestyle changes (e.g., drink less fluids, take naps during the day because it is impossible to sleep through the night) to try to ameliorate their symptoms. Thus, the AUA-SI score may not correlate with the size of the prostate as assessed by digital rectal exam, the decrease in peak urinary flow rate, or the degree of bladder neck obstruction on cystoscopy, which are usual objective findings in patients with BPH.

3. ***A patient has an IIEF score of 15. Is this consistent with the patient's complaint of erectile dysfunction?***

   ANSWER: The IIEF is a validated self-assessment questionnaire used to assess the severity of erectile dysfunction. The score ranges from 10–25. A score of 15 is consistent with mild-to-moderate erectile dysfunction. The IIEF tool can be used to monitor a patient's response to treatment.

4. ***What effect do 5α-reductase inhibitors have on PSA? What precautions should be taken in patients to preserve the usefulness of PSA as a tumor marker in patients taking 5α-reductase inhibitors?***

   ANSWER: 5α-reductase inhibitors decrease PSA levels by approximately 50% after six months of continuous use. Therefore, it is recommended to establish a baseline PSA prior to the start of a 5α-reductase inhibitor. If the PSA does not decrease by 50% or it increases after six months of 5α-reductase inhibitor use, the patient's adherence to drug therapy should be evaluated. If the patient is taking the medication as prescribed, then the patient should be evaluated for diseases that can increase PSA, including prostate cancer.

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QUICKVIEW | Testosterone

PARAMETER	DESCRIPTION	COMMENTS
Reference range		
Adult, males	280–1100 ng/dL (9.7–38.17 nmol/L)	Normal range exhibits variability laboratory-to-laboratory This is largely due to the immunoassay method, which is commonly used It is recommended that each laboratory establish its own normal range
Critical values	<200–230 ng/dL (<6.94–7.98 nmol/L) is generally associated with symptomatic hypogonadism ≤50 ng/dL (≤1.74 nmol/L) is associated with surgical or medical castration for prostate cancer Residual serum testosterone levels reflect continuing adrenal androgen production	Extremely high or low values should be reported quickly
Inherent action?	Yes	Exerts different physiologic effects at different stages of life in males (Table 23-1)
Location		
Production	Testosterone is produced in the testes	The testes produce 90% of circulating androgen; the rest is produced by the adrenal glands
Storage	It is not stored	
Secretion/excretion	Testosterone is activated to DHT intracellularly in some target tissues by 5α-reductase; in adipose tissue, excess testosterone is converted to estrogen	In some target tissues (e.g., brain) testosterone is active; in other target tissues (e.g., prostate and scalp) testosterone must be activated to DHT to exert an effect; peripheral conversion of testosterone to estrogen results in gynecomastia
Causes of abnormal values		
High	Hyperthyroidism, adrenal tumors, adrenal hyperplasia, testicular tumors, precocious puberty, anabolic steroids, excessive testosterone supplementation	
Low	Primary or secondary hypogonadism, late-onset hypogonadism, primary or secondary hypopituitarism, Klinefelter syndrome, orchiectomy, traumatic injury to testicles, mumps, maldescent of testicles, hepatic cirrhosis, prolactinoma, high-dose corticosteroids, LHRH antagonists, LHRH agonists, estrogens, cytotoxins, high dose ketoconazole	
Signs and symptoms		
High level	Increased libido, mood swings	
Low level	Absent or depressed libido, lack of energy, decreased sense of well being, erectile dysfunction, gynecomastia, small testicles, decreased body hair, decreased muscle strength, visceral obesity, hot flashes	

QUICKVIEW | Testosterone (cont'd)

PARAMETER	DESCRIPTION	COMMENTS
After event, time to…		
Initial evaluation	After orchiectomy, serum testosterone levels decrease to ≤50 ng/dL (≤1.74 nmol/L) in several hours	Orchiectomy is indicated for symptomatic management of metastatic prostate cancer
Peak values	After depot LHRH superagonist injection, serum testosterone levels decrease to ≤50 ng/dL (≤1.74 nmol/L) in 2–3 wk	LHRH superagonists are alternatives to orchiectomy for symptomatic management of metastatic prostate cancer
Normalization	With testosterone supplementation for late-onset hypogonadism, an adequate clinical trial is 3 mo in length After supplementation is started, serum testosterone should be repeated every 3–4 mo during the first year A low baseline serum testosterone level should return to the normal range with adequate supplementation	Depending on the dosage formulation of testosterone supplement, supraphysiologic serum testosterone concentrations may be produced after administration This occurs with intramuscular depot injections In contrast, with other dosage formulations (e.g., testosterone transdermal patches or buccal patch systems), only physiologic serum testosterone concentrations are produced after drug administration The clinical significance of this difference is not known
Causes of spurious results	Excessive testosterone supplementation	
Additional information	Testosterone bound to SHBG is inactive; therefore, conditions which significantly alter the concentration of SHBG can increase or decrease the concentration of free testosterone, which is physiologically active (refer to Table 23-2 for a listing of such conditions)	In such patients, a calculated or measured free or bioavailable testosterone level would be preferred over a total serum testosterone level

DHT = dihydrotestosterone; LHRH = luteinizing hormone–releasing hormone; SHBG = sex hormone–binding globulin.

QUICKVIEW | PSA[a]

PARAMETER	DESCRIPTION	COMMENTS
Reference range		
Adult, males	<4 ng/mL (<4 mcg/L)	This cutoff value misses 27% of patients with organ-confined prostate cancer; as a result, some experts recommend using age-related normal ranges (Table 23-19), % fPSA, or PSA velocity instead
Critical values	≥10 ng/mL is highly suggestive of prostate cancer	Extremely high values should be reported to the physician quickly
Inherent action?	Yes	Responsible for liquefying semen after ejaculation
Location		
Production	PSA is produced by the prostate	Blood levels of PSA are usually very low; however, in patients with prostate cancer or other diseases of the prostate, the normal prostatic architecture of ducts is not intact Instead of passing out of the body, PSA enters the bloodstream, which results in elevated blood levels
Storage	It is not stored	
Secretion/excretion	PSA normally passes out of the body in the ejaculate; it liquefies semen	
Causes of abnormal values		
High	Prostate cancer	Noncancerous causes of high results include BPH, prostatitis, prostate trauma, prostate surgery, acute urinary retention, ejaculation, exercise bicycling, exogenous testosterone supplements
Low	Low laboratory results are normal	
Signs and symptoms		
High level	This disease is commonly asymptomatic until the prostate cancer is large enough to cause voiding symptoms, or until the tumor has metastasized; in the latter case, the patient may complain of bone pain, shortness of breath, or leg weakness due to bone, lung, or spinal cord metastases, respectively	
Low level	Not applicable	
After insult, time to…		
Initial evaluation	After prostate manipulation, the PSA levels will increase within hours and remain elevated for the duration of the prostatic inflammation; for example, after prostate massage, PSA may return to the normal range within days, whereas after transurethral prostatectomy, it may take weeks	
Peak values	No maximum value	
Causes of spurious results		
	As patients age, PSA normally increases BPH and organ-confined prostate cancer show overlap in PSA levels Refer to Table 23-18 for other conditions that increase or decrease PSA	

BPH = benign prostatic hyperplasia; PSA = prostate specific antigen.

[a] Percentage of fPSA, PSA velocity, and PSA density are additional types of PSA tests that are used to improve the usefulness of PSA as a tumor marker for prostate cancer screening and to monitor treatment response.

GLOSSARY

1,25 dihydroxycholecalciferol—the most potent form of vitamin D, which is a result of both hepatic and renal activation of a precursor. It enhances intestinal absorption of calcium, increases parathyroid hormone-induced bone resorption, and enhances calcium reabsorption in the proximal renal tubules. Overall, vitamin D is important for maintaining serum calcium levels in the normal range. Also known as *calcitriol.*

5α-reductase—an intracellular enzyme that converts testosterone to the active metabolite dihydrotestosterone. Two types of 5α-reductase exist. Type I is found mostly in the skin, liver, and sebaceous glands. Type II is found mostly in urogenital tissue, including the prostate, and hair follicles.

6-acetylmorphine—an active metabolite of heroin. When present at a concentration that meets or exceeds the federal cutoff in a urine drug test, it confirms that the patient was recently using heroin.

Accuracy—refers to how close the test result or mean measurement is to the true value of the analyte in a specimen or to the number or percentage of true results relative to the total number of samples tested. Also referred to as *bias.*

Achiral—a drug that exists in only one form such that the molecules are superimposable on their mirror images.

Achlorhydria—a disorder resulting in insufficient or absence of hydrochloric acid from gastric secretion. Among the conditions associated with achlorhydria is pernicious anemia.

Acrodermatitis enteropathica—a rare inherited disorder of infants and young children characterized by skin eruptions around the mouth and other body orifices, alopecia, and diarrhea. The disorder is caused by zinc deficiency.

Acromegaly—excessive pituitary gland production of growth hormone, which can lead to giantism if it occurs in adolescents or to thickening of the skin and enlargement of hands and feet if it occurs after puberty.

Action potential—changes in electrical potential across muscle or nerve cell when triggered by an appropriate stimulus leading to muscle cell contraction or transmission of an electrical signal by a nerve cell.

Acute-phase reactants—a class of plasma proteins whose concentration increases in response to inflammatory stimuli, such as tissue injury or infection. Also known as *acute-phase proteins.*

Addison disease—a disorder caused by chronic insufficiency of adrenal cortisol and, sometimes, aldosterone. Symptoms include extreme weakness, dizziness on standing, nausea, vomiting, chronic diarrhea, weight loss, salt craving, slow sluggish movement, and development of dark patches on the skin. The disease is treated with exogenous administration of corticosteroid (e.g., prednisone, hydrocortisone) and aldosterone (e.g., fludrocortisone).

Adipsia—absence of thirst.

ADME—refers to absorption, distribution, metabolism, and excretion of drugs, which are all related to pharmacokinetic handling of drugs by the human body.

Agglutination—the clumping or aggregation of cells (e.g., erythrocytes) in a solution.

Airway resistance—this reflects the degree of ease with which air can pass through the airways. It is expressed as the change in pressure divided by the change in flow.

Allele—an alternate form of the gene that is located at a particular chromosomal location. One allele is inherited from the mother and one from the father.

α-fetoprotein (AFP)—a glycoprotein produced by the liver, gastrointestinal tract, and fetal yolk sac. Elevated serum levels may occur in patients with hepatocellular carcinoma, nonseminomatous germ cell tumors, and cancers of the pancreas, stomach, lung, and colon. For this reason, AFP is used as a tumor marker.

α1-acid glycoprotein—an acute-phase reactant that is a protein produced by the liver. It circulates in the plasma, constitutes 1–3% of circulating plasma proteins, and serves as a carrier for basic drugs.

Amenorrhea—absence of menstrual bleeding, which may be primary or secondary.

Amylin—a peptide hormone, cosecreted with insulin, that slows gastric emptying after eating, decreases gastrointestinal glucose absorption, and promotes satiety. Amylin helps reduce postprandial increases in serum glucose levels.

Analyte—the substance measured by the laboratory assay.

Androstenedione—an androgen produced by the adrenal glands, testes, and ovary that is converted to estrone in both females and males. High androstenedione levels in men can result in feminization.

Angiotensin II—an octapeptide that has two physiologic effects: it is a potent arteriolar vasoconstrictor, and it also stimulates the release of aldosterone, which causes renal sodium retention. Angiotensin II also increases the release of antidiuretic hormone and adrenocorticotropic hormone.

Anion gap—a calculated value that is used to identify potential causes of metabolic acidosis. The anion gap is estimated by subtracting the sum of serum chloride and venous bicarbonate concentrations from the serum sodium concentration.

Anisocytosis—variability in the size of erythrocytes (red blood cells).

Antibiogram—a cumulative report describing the in vitro antimicrobial susceptibility results of the most common bacterial strains isolated at a particular institution/healthcare setting during the time period of the report (usually annually).

Anticitrullinated protein antibodies—antibodies that bind to the nonstandard amino acid citrulline and are highly specific for rheumatoid arthritis when present in serum. Also known as *anti-citrulline antibody* or *citrulline antibody*.

Antidiuretic hormone—a hormone that regulates renal handling of free water and enhances reabsorption of water at the collecting duct portion of the renal tubule. It is secreted by the hypothalamus in response to hypovolemia, thirst, increased serum osmolality, and angiotensin II. Also known as *arginine vasopressin*.

Antineutrophil cytoplasmic antibodies (ANCAs)—antibodies that are directed against neutrophil cytoplasmic antigens. Testing for ANCAs is important for diagnosis and classification of various forms of vasculitis.

Antinuclear antibodies (ANAs)—autoantibodies that are directed against components of the cell nucleus, such as DNA, RNA, and histones.

Antiphospholipid antibodies—antibodies that react with proteins in the blood bound to phospholipid. Antiphospholipid antibodies interfere with the normal function of blood vessels by causing narrowing and irregularity of the vessel, thrombocytopenia, and thrombosis. Examples of these antiphospholipid antibodies include lupus anticoagulant and anticardiolipin antibodies.

Apophysis—an offshoot.

Apoptosis—noninflammatory cell death via autolysis.

Aquaporin—a water channel in the collecting duct that facilitates water reabsorption from the tubular lumen back into the bloodstream. Formation is stimulated by antidiuretic hormone.

Areflexia—absence of reflexes, which usually indicates a neurologic problem.

Arthrocentesis—a procedure in which a sterile needle and syringe are used to aspirate fluid from a joint.

Asherman syndrome—the development of intrauterine scar tissue after intrauterine surgery, which can lead to amenorrhea.

Atrial natriuretic factor—a vasodilatory hormone synthesized and primarily released by the right atrium. It is secreted in response to plasma volume expansion as a result of increased atrial stretch and results in a global down regulation of renin, aldosterone, and antidiuretic hormone. A net increase in sodium excretion is achieved. Also known as *atrial natriuretic peptide*.

B-RAF—a protein that stimulates cell growth. Mutations of B-RAF proteins are associated with cancer and birth defects. Mutated B-RAF proteins have elevated kinase activity. Vemurafenib is a serine-threonine protein kinase B-RAF inhibitor indicated for patients with advanced melanoma harboring the B-RAF gene. B-RAF is made by a human gene known as BRAF. Also known as *serine-threonine protein kinase.*

Bartter syndrome—a syndrome that presents with hypokalemia, alkalosis, increased renin and aldosterone, and normal-to-low blood pressure. It is a cause of secondary hyperaldosteronism.

Benzoylecgonine—a major metabolite of cocaine.

β-lactamases—enzymes produced by some bacteria that are capable of breaking of the chemical ring structure of, deactivating antibacterial properties, and mediating resistance to selected β-lactam antibiotics.

Bias—refers to how close the test result is to the true value of the analyte in a specimen or to the number or percentage of true results relative to the total number of samples tested. Also referred to as *accuracy.*

Biomarker—an objectively measured indicator of normal biological or pathogenic processes or pharmacologic responses used to diagnose and stage disease, assess disease progress, or assess response to therapeutic interventions.

Biosensor system—a bioreceptor molecule, which recognizes a target analyte and either generates a specific molecular species or results in a physiochemical change that can be measured by electrochemical methods.

Blastoconidia—a bud produced by asexual reproduction of fungus.

Brain natriuretic peptide (BNP)—a peptide that is principally produced and secreted by the ventricles of the brain. An increase in blood volume or pressure enhances BNP secretion, which increases natriuresis (renal sodium excretion) and to a lesser extent, diuresis. Also known as *B-type natriuretic peptide.*

Bronchial alveolar lavage—a procedure in which a bronchoscope is inserted into the lumen of the airways and sterile normal saline solution is flushed into the airways and then removed by aspiration. The solution is then sent for cellular and chemical analysis.

Bulimia—an illness characterized by periods of overeating or binging followed by purging and vomiting.

C-peptide—proinsulin, a precursor of insulin, is comprised of C-peptide and insulin. C-peptide must be cleaved from insulin for insulin to function. Elevated C-peptide levels in the blood stream are consistent with increased insulin levels.

Cachectic—a term used to describe a patient who has a wasting syndrome characterized by severe weight loss, muscle atrophy, and fatigue. The patient is unable to or does not want to eat. This is commonly used to describe patients with cancer, AIDS, or other chronic medical disorders.

Calcidiol—25-hydroxycholecalciferol, which is also known as *calcifediol*. This is a form of vitamin D with intermediate activity that has been activated by the liver but still needs to be activated by the kidneys to be most active.

Calcitonin—a hormone secreted by the C-cells of the thyroid gland. It inhibits osteoclastic activity, thereby inhibiting bone resorption. It also decreases calcium reabsorption in the renal proximal tubules.

Calcitriol—1,25 dihydroxycholecalciferol, which is the most active form of vitamin D. It has completed two activation steps: one by the liver and the other by the kidneys.

Calcium-phosphorus product—the multiplication product of serum calcium and phosphorus concentrations, expressed as mg/dL. Insoluble calcium-phosphorus precipitates are likely to be formed in soft tissues or as stones in the genitourinary tract when the product is high.

In patients with chronic kidney disease, dietary restriction and pharmacotherapy are used to maintain the calcium–phosphorus product at 50 or less.

Capillary puncture—blood sampling method for premature neonates, neonates, and young infants who have small or inaccessible veins. Blood sampling is done at the heel, fingertip, or great toe. Also known as *microcapillary puncture* or *skin puncture*.

Carcinoembryonic antigen (CEA)—a protein that is normally found in fetal intestine, pancreas, and liver. Elevated serum levels of CEA are found in patients with colon, breast, gastric, thyroid, or pancreatic cancer. For this reason, CEA is used as a tumor marker.

Cast—masses of glycoproteins that conform to the shape of the renal tubular lumen. Casts are detected by microscopic evaluation of the urine. The cellular composition of some casts are suggestive of the presence of various types of renal disorders.

Central or centripetal obesity—a term for when a patient has fat accumulation around the waist, abdomen, and hips.

Chain-of-custody—a process used to collect urine specimens that safeguards the specimen from purposeful adulteration or dilution of the specimen. A chain-of-custody ensures that the specimen is always in the custody of a person responsible for ensuring the integrity of the urine specimen.

Cholestasis—a deficiency of the excretory function of the liver.

Cholesterol—a substance of dietary origin or synthesized in the liver and intestines that serves as a structural component of cell wall membranes and is a precursor for the synthesis of steroid hormones and bile acids.

Chromatography—a common laboratory separation technique that identifies and quantifies the solutes in a solution based on their differential distribution between mobile and stationary phases. The mobile phase generally refers to the dissolution of the laboratory sample in a fluid. The stationary phase is a substance that is fixed in place during the separation procedure. The separation is based on the partition coefficient of the analyte. There are several types of chromatography, including thin layer, gas, and high-performance liquid.

Chvostek sign—a sign of hypocalcemia and tetany. Tapping the facial nerve on one side of the face results in a facial twitch or grimace on the same side.

CKD-EPI collaboration equation—an updated variation of the MDRD equation developed to better identify patients with chronic kidney disease that would benefit from the specialized care of a nephrologist.

CLIA-waived test—the U.S. Food and Drug Administration classifies these laboratory test procedures as having a low level of complexity to perform and a low potential risk to produce erroneous results or pose no reasonable risk of harm to the patient if performed incorrectly. These tests typically are performed in community pharmacies or ambulatory care clinics, which are not subject to regular inspections, personnel requirements, or proficiency tests once the latter have obtained a CLIA Certificate of Waiver from the Centers for Medicare & Medicaid Services.

Cluster of differentiation—refers to a molecule or antigen on a cell's surface to which a monoclonal antibody can bind. These are used as markers to distinguish one cell type from another.

Coagulation—process by which blood forms clots.

Cobalamin—vitamin B_{12}. Cyanocobalamin is a synthetic type of cobalamin, which is a commercially available medication.

Cockcroft-Gault equation—an equation that is used to estimate creatinine clearance in patients with stable renal function. The equation requires that the patient's gender, age, total body weight, and serum creatinine be known.

Codon—three base pairs that specify an amino acid. Because of redundancy in the genetic code, a change in one base pair may or may not change the amino acid coded by the codon.

Colonization—the presence of microorganisms, including potential pathogens, at a body site (i.e., oropharynx, skin, colon, vagina, surfaces of wounds) that are not causing infection.

Complement—a cascade system of at least 60 different plasma proteins that interact to provide a defense mechanism against microbial invaders and serve as an adjunct or "complement" to humoral immunity. The complement cascade can be activated via the classical, alternative, or lectin pathways.

Compliance (of the lung)—the ability of the lungs to expand and fill with air during inhalation.

Congenital adrenal hyperplasia—a rare inherited disease of the adrenal glands in which cortisol and aldosterone production are impaired, but androgen production is excessive.

Constitutive enzyme—an enzyme that is produced continuously although it is not needed.

Corpora cavernosa—one of two channels on the dorsal side of the penis, which is comprised of sinusoidal tissues. During a penile erection, the sinuses fill with arterial blood. In the flaccid state, the sinuses are empty.

Corpus luteum—remnants of the ovarian follicle that result after the follicle expels the ovum into the fallopian tube.

Corrected serum calcium concentration—in patients with low plasma binding of calcium, often as a result of hypoalbuminemia, the "effective" serum calcium concentration must be assessed. The measured serum calcium concentration has to be corrected according to the serum albumin concentration. The corrected serum calcium concentration better reflects the amount of physiologically active calcium, which is the free (unbound) moiety.

Costochondral junction—the point where the ribs connect to the cartilage in the sternum (breast bone). Palpable enlargement of the costochondral junctions is called the rachitic rosary sign and is compatible with the diagnosis of rickets.

Crack—a freebase form of cocaine that produces an intense high when smoked. Also known as crack cocaine.

C-reactive protein—plasma protein associated with the acute-phase response to injury or infection. The precise physiologic function of c-reactive protein is unknown, but it is known to participate in activation of the complement pathway and interact with cells in the immune system.

Creatine kinase—an enzyme found in skeletal muscle, heart muscle, and brain. It stimulates the transfer of high-energy phosphate groups and converts adenosine triphosphate to adenosine diphosphate, and vice versa. Elevated circulating levels are associated with musculoskeletal injury, intramuscular injections, or some medications. Creatine kinase is also known as *creatine phosphokinase.*

Creatinine—an endogenous substance produced by muscle cells. The production rate varies little day-to-day in patients with stable kidney function. Creatinine is freely filtered at the glomerulus with little reabsorbed or secreted. It is commonly used for assessing kidney function.

Creatinine clearance—a practical method of assessing kidney function to monitor kidney disease or dose medication. It can be derived by measuring creatinine concentration via a urine collection or by using the serum creatinine concentration in the Cockcroft-Gault equation, or some other validated equation.

Crigler-Najjar syndrome—a rare genetic disorder in which bilirubin cannot be conjugated by the liver. If not treated, bilirubin accumulates in the blood stream resulting in kernicterus.

Critical value—a result far enough out of the reference range that it indicates impending morbidity.

Cryptorchidism—the failure of one or both testicles to descend through the inguinal canal into the scrotum after birth. As a result, the undescended testicle is at high risk for twisting on its spermatic cord, decreasing spermatogenesis, and developing testicular cancer.

Cyclooxygenase—an enzyme found in platelets responsible for converting arachidonic acid to thromboxane (which is an arterial constrictor and causes platelet aggregation) and prostacyclin (which is a vasodilator and inhibits platelet aggregation).

Cystatin C—a protease inhibitor that is filtered by the glomerulus but not reabsorbed or secreted. Cystatin C serum concentrations are used in various formulae to estimate glomerular filtration rate.

Cystic fibrosis—a genetic disorder that primarily affects the lungs and gastrointestinal tract. Patients with cystic fibrosis produce very thick mucus that can clog the airways of the lungs and cause severe lung infections that can be life-threatening. The mucus also obstructs the outflow tract of the pancreas and stops pancreatic enzymes from breaking down food. The sweat of patients with cystic fibrosis contains a high amount of sodium chloride.

D-dimer—a neoantigen formed when plasmin digests fibrin. When present on blood testing, D-dimer indicates the presence of thrombosis.

Dead space (of the alveoli)—alveolar dead space results when there is a ventilation-perfusion mismatch. That is, alveoli are ventilated, but perfusion is absent or inadequate for effective gas exchange of oxygen and carbon dioxide. Alveolar dead space also may result when areas of the lung are well perfused but not ventilated.

Dematiaceous fungi—a type of fungi that produces darkly pigmented hyphae or spores.

Depolarization—an electrical phenomenon that represents the decrease in the differential ionic charges across muscle or nerve cell membranes from the resting state to the excited state. The intracellular space becomes more positively charged than the extracellular space leading to cellular activation and contraction.

Dermatophytes—a term for a group of fungi, including *Microsporum, Epidermophyton*, and *Trichophyton*, that cause skin infections.

Diabetes insipidus—a condition in which the kidneys are not able to reabsorb water (back into the body). Central diabetes insipidus is caused by a lack of pituitary secretion of antidiuretic hormone (ADH). Nephrogenic diabetes is caused by failure of the kidneys to respond to ADH. If the patient is unable to drink enough fluids to replace urinary water losses or is not treated for diabetes insipidus, the patient will become dehydrated.

Diabetic ketoacidosis—acute, severe, life-threatening presentation of insulin deficiency that is associated with dehydration, abdominal pain, tachycardia, orthostatic hypotension, lethargy, or coma. The patient has severe hyperglycemia, ketosis, and metabolic acidosis. Because of insulin deficiency, the body is unable to use glucose as an energy source and burns fat instead. The resulting ketosis is responsible for many of the resulting symptoms and signs of disease.

Diffusion—the process in which gases in the alveoli equilibrate from areas of high concentration to areas of low concentration.

DiGeorge syndrome—a genetic disorder associated with congenital heart disease, defects of the palate, learning disabilities, malfunctioning parathyroid glands, and thymus aplasia. Also known as *22q11.2 deletion syndrome.*

Dihydropyrimidine dehydrogenase—an enzyme that metabolizes 5-fluorouracil. A genetically-mediated enzyme deficiency is associated with increased 5-fluorouracil toxicity.

Direct bilirubin—formed when the liver conjugates bilirubin by linking it to glucuronic acid. This creates a water soluble form of bilirubin, which is excreted into bile and eliminated in feces. Direct bilirubin can also be excreted in urine.

Disk diffusion method—a widely used method to determine a bacteria's sensitivity to various antibiotics. Commercially prepared filter paper disks containing a fixed concentration of an antibiotic are placed on solid media agar plates inoculated with a standardized inoculum of the infecting organism. As antibiotic diffuses from the disk into the agar, it creates zones of inhibition that correlate with the minimum inhibitory concentrations of the antibiotics against certain organisms. Also known as the *Kirby Bauer method.*

Dubin-Johnson syndrome—an autosomal recessive disease in which hepatocytes fail to secrete conjugated bilirubin into bile. As a result, patients develop high serum levels of conjugated bilirubin and mild jaundice.

Dynamic spirometry—a pulmonary breathing test that is based on time and, therefore, is more dependent on flow and "forced."

Dysgeusia—an impaired sense of taste.

Dyslipidemia—abnormal concentrations of any lipoprotein type.

Dyspareunia—painful intercourse.

Eclampsia—a condition that occurs in pregnant women when preeclampsia is not treated. In addition to the symptoms of preeclampsia, women may experience seizures and coma. Eclampsia is a serious condition, as death of the mother and baby may occur.

EGFR-TKI—epidermal growth factor receptor tyrosine kinase inhibitor. An example drug is erlotinib. Increased sensitivity to EGFR-TKIs has been linked to the presence of EGFR activating mutations in the tumor, mostly exons 18 and 21 of the EGFR gene. EGFR testing has been used for non-small-cell lung cancer and other tumors.

Electrocardiography—the recording of the electrical activity of the heart on an electrocardiogram.

Electrophoresis—a common laboratory technique involving separation of charged solutes or particles, which are based on size or shape in a solution or support medium (e.g., agarose or polyacrylamide gel). There are several types of electrophoresis, including gel, two-dimensional, capillary, and capillary zone.

Enantiomer—one of a pair of nonsuperimposable mirror image molecules. Enantiomers of a chiral drug may have different effects.

Enterocutaneous fistula—an abnormal connection between the stomach or intestine with the abdominal skin. As a result, contents of the gastrointestinal tract leak out through the skin. This is an example of an external fistula.

Enterohepatic recycling—a process by which medications or other substances are carried by bile into the intestine, then reabsorbed from the intestines and delivered to the liver for metabolism and secretion in bile, which is stored in the gall bladder. From there, the medication or other substances can be secreted back into the intestine through the biliary system.

Enthesitis—an inflammation of the sites where tendons or ligaments attach to bone.

Enzyme-linked immunosorbent assay—a type of enzyme immunoassay in which the antigen is complexed to an enzyme. After the antigen binds to the antibody in the sample, the assay measures the amount of enzyme activity that is used to quantitate the amount of antigen.

Enzyme-multiplied immunoassay—a type of enzyme immunoassay commonly used to measure the serum drug levels in a patient. It is based on adding a known quantity of antidrug antibody and drug bound to an enzyme to a patient's laboratory sample that contains free drug. The free drug and the enzyme-linked drug compete for binding with the antidrug antibody. When the antibody binds to the enzyme-linked drug, enzyme activity is inhibited. The serum drug concentration is determined from the amount of active enzyme remaining.

Epidermal growth factor receptor—a receptor that supports tumor growth when activated. The gene that encodes for EGFR is most commonly found in adenocarcinoma of the lungs in nonsmokers. Also known as *EGFR, HER1,* or *C-Erb B1.*

Epigenetics—modifications of the genome (e.g., DNA methylation or histone deacetylation) that may result in different genetic or phenotypic expressions. Epigenetic changes are due to external or environmental factors and are not associated with changes in the underlying DNA sequence of the organism.

Epsilometer test—a test that evaluates the activity of numerous concentrations of an antibiotic against bacteria, which is causing an infection. The results of the Etest are reported as the MIC of the antibiotic against the infecting bacteria, which are used with the Clinical and Laboratory Standards Institute established interpretative criteria to categorize the bacteria's response as sensitive, intermediate (sensitivity), or resistant. Also known as the *Etest.*

Erythropoiesis—a process of producing red blood cells.

Esophageal varix—an engorged, superficial vein in the lumen of the esophagus (plural is varices).

Euvolemic—refers to patients with normal plasma volume.

Exon—sequence of a gene that is translated into mRNA and protein.

Extracellular water compartment—consists of interstitial water and circulating plasma volume. The extracellular water compartment and intracellular water compartment comprise total body water.

Extrahepatic cholestasis—anatomic obstruction of macroscopic bile ducts.

Fastidious—an organism with complex or excessive nutritional requirements, which makes it difficult to grow it in the laboratory. Such an organism is generally referred to as fussy or demanding.

FE_{NA} or fractional excretion of sodium—percent of filtered sodium that is ultimately excreted in the urine.

Ferritin—the intracellular form of stored iron. Iron is bound to a storage protein.

Fibrinolysis—a mechanism by which formed thrombi are lysed through the dissolution of fibrin to prevent excessive clot formation and vascular occlusion.

Fibroids—the most common benign tumor of the uterus.

Fibromyalgia—a syndrome of pain, fatigue, sleep disturbances, and other medical problems. According to the American College of Rheumatology, an individual must have a history of chronic widespread pain and tenderness at 11 or more of 18 specific tender-point sites on physician examination.

Fingerstick—a method of obtaining venous blood by pricking the fingertip with a lancet.

First order absorption or elimination rate—The absorption or elimination rate of a drug from the body proceeds at a rate that is dependent on the plasma drug concentration. For example, as the dose increases, plasma concentrations increase, and the rate of elimination increases.

Fistula—an abnormal communication, opening, or passage from one hollow organ or abscess to another organ or to the skin. This could be due to infection, congenital malformation, or other disease.

Flow cytometry—a laboratory technique that measures the properties of cells as they move or flow in a liquid suspension stream. A laser is used to count or sort thousands of cells per second.

Fluorescence in situ hybridization (FISH)—a laboratory technique used to look at genes or chromosomes in cells and tissues. Pieces of DNA that contain a fluorescent dye are made in the laboratory and used as probes. These DNA probes light up when they bind to specific genes or chromosomes and are viewed under a microscope with ultraviolet light.

Fluorophore—a fluorescent molecule that can reemit light upon exposure to light. Fluorophores are often used as tracers or dyes for staining laboratory samples.

Follicular phase—early portion of the menstrual cycle during which the ovarian follicle matures.

Forced vital capacity—total volume of air measured in liters that is forcefully and rapidly exhaled in one breath.

ft>MIC—time that free serum antimicrobial concentrations are above the organism's minimum inhibitory concentration.

Fusiform—shaped like a spindle; wide in the middle and tapered at each end.

Galactorrhea—the secretion of a milky discharge from the breast other than when breastfeeding.

γ-hydroxybutyrate—an illicit substance that is a central nervous system depressant. Also known as *GHB* or the *date rape drug*.

Gastroschisis—a defect in the wall of the abdomen, which occurs during fetal development (i.e., a congenital malformation or birth defect). It allows the intestines (and sometimes other organs) to develop outside of the abdominal cavity. Having the internal organs outside of the abdominal wall will increase insensible water loss.

Genotype—the inherited genetic makeup of an organism.

Geophagia—the practice of eating dirt, earth, clay, or chalk.

Gestational age—the number of weeks from the first day of the mother's last menstrual period until the birth of the baby.

Gestational diabetes—the onset of diabetes mellitus during pregnancy.

Gilbert syndrome—a rare, hereditary deficiency of glucuronyltransferase, which normally conjugates bilirubin in the liver. As a result, patients develop high serum levels of unconjugated bilirubin and jaundice.

Glabrous—refers to hairless parts of the body, including palms of hands and soles of feet.

Glucagon—a peptide hormone secreted by the pancreas, which increases serum glucose concentration by stimulating gluconeogenesis and glycogenolysis in the liver.

Gluconeogenesis—the process when the liver produces glucose from protein or fat sources.

Glycogenolysis—the breakdown of glycogen in muscle and liver to glucose and glucose-1-phosphate.

Glycosylated hemoglobin—glucose combines irreversibly with hemoglobin in red blood cells to form glycosylated hemoglobin. Increased glycosylated hemoglobin serum levels are indicative of poor long-term glucose control. Also known as *glycated hemoglobin*.

Goitrogenic—refers to a substance (e.g., food or medication) that suppresses thyroid gland function. As a result, TSH levels increase, which stimulates growth of the thyroid gland. If severe and untreated, the large goiter can cause obstruction of the trachea or esophagus.

Granulocyte—a category of white blood cell that has phagocytic activity. Granulocytes include neutrophils, eosinophils, and basophils. Also known as *polymorphonuclear leukocyte.*

Granulosa cells—in the ovarian follicle, these cells produce estradiol before ovulation and progesterone after ovulation.

Haplotype—a set of alleles from a single chromosome that tends to be inherited as a unit.

Hapten—a small molecule that can stimulate an immune response, usually when it is attached to a protein.

Hashimoto thyroiditis—a chronic progressive thyroid disease where functioning thyroid tissue is replaced by lymphoid or scar tissue. The patient may develop a goiter and has hypothyroidism.

Heelstick—capillary puncture of the heel; a blood drawing technique used in pediatric patients with small or inaccessible veins. It is the blood sampling method of choice for premature neonates, neonates, and young infants.

Hemarthrosis—bleeding into a joint space.

Hematopoiesis—a process by which bone marrow produces red blood cells, white blood cells, and platelets.

Hemochromatosis—an excessive accumulation of iron in the body, typically in the liver, heart, and endocrine glands.

Hemoconcentration—an abnormally high concentration of cells or other solutes due to a decreased amount of fluid in the bloodstream.

Hemolysis—the destruction of red blood cells with abnormal release of hemoglobin; can occur in vitro or in vivo.

Hemosiderin—a storage form of iron in macrophages. The iron in hemosiderin is poorly available for use by the human body.

Hemostasis—a complex relationship among substances that promotes clot formations, inhibits coagulation, and dissolves formed clots.

Hepatic encephalopathy—a diffuse metabolic dysfunction of the brain, which may occur in acute or chronic liver failure. Clinically, it ranges from subtle changes in personality to coma and death.

Hepatitis—a histologic pattern of inflammation of hepatocytes.

Hepatojugular reflux—a physical exam finding associated with congestive heart failure or tricuspid regurgitation. Gentle pressure on the liver causes distention of the jugular vein in the neck.

HER2—human epidermal growth factor receptor 2. The protein is overexpressed in approximately 25% of breast cancers. When semiquantitative tests show that HER2 overexpression is present, the patient is more likely to respond to trastuzumab.

Hirsutism—an excess hair growth in women due to excessive androgen stimulation. Excessive hair may appear in sideburn area, chin, upper lip, periareolar area of breast, chest, lower abdominal midline, and thighs.

Histone antibodies—a type of antinuclear antibody that is directed at DNA-protein complexes comprising part of chromatin. Histone antibodies are present in virtually all cases of drug-induced lupus.

Home testing—patient-directed diagnostic and monitoring tests performed by the patient or family member at home.

Human chorionic gonadotropin (hCG)—a glycoprotein that is normally produced by the placenta during pregnancy. Also, elevated hCG levels are seen in patients with some tumors of the testes and ovaries. For this reason, serum levels of hCG are used as a tumor marker.

Hydatidiform mole—a benign uterine tumor that presents as a mass of cysts.

Hyperosmolar hyperglycemic state—a state in which patients have severe hyperglycemia, generally >600 mg/dL, but not ketosis. Hyperglycemia causes glycosuria and dehydration. Water shifts from the intracellular to vascular compartment to compensate. This commonly occurs in type 2 diabetes mellitus, when the patient is stressed by a concurrent medical illness (e.g., infection). Also known as *hyperosmolar hyperglycemia nonketotic state.*

Hypha—referring to fungi; this is a branching tube-like filament.

Hypogeusia—the diminished ability to taste food.

Hyposmia—a decreased sense of smell.

Icterus—yellow discoloration of the sclerae and skin associated with hyperbilirubinemia. Also known as *jaundice*.

Ileus—an obstruction of the bowel that leads to nausea, vomiting, and abdominal pain. It could be due to a physical obstruction or absence of peristalsis. Also known as *paralytic ileus*.

Immunoassay—a common laboratory technique based on a reaction between an antigenic determinant and a labeled antibody.

Immunohistochemistry (IHC)—the process of obtaining tissue from a biopsy and fixing it onto a glass slide. Antibodies to the antigen thought to be in the biopsy specimen are added and bind to the antigen. The antibodies that are bound to the antigen stain the biopsy and then are read by a pathologist to determine the amount of staining present in the biopsy specimen.

Incretin—gut hormones that enhance insulin secretion when serum glucose levels rise after meals. Two major incretins are glucagon-like peptide and glucose-dependent insulinotropic peptide.

Indirect bilirubin—unconjugated bilirubin that is water insoluble and must be converted to direct bilirubin by the liver in order to be excreted.

Infant—refers to a baby that is one month to one year of age.

Infarction—the death of part or whole of an organ secondary to obstruction of blood flow by a blood clot (thrombus) or an embolus in the supplying artery.

Informatics—the use of collected data for the purposes of problem solving and healthcare decision-making.

Inotropic—related to the contraction of heart muscle (e.g., positive inotropic agents increase the force of contractions of the heart muscle).

Intermediate (I)—interpretive category for in vitro susceptibility testing of bacteria where the resulting minimum inhibitory concentration (MIC) is equivocal (i.e., MIC is higher than those interpreted as susceptible but lower than those interpreted as resistant). The organism/infection may be eradicated if the antimicrobial agent achieves high concentrations at the site of infection or maximum doses of the antimicrobial agent are utilized.

International normalized ratio (INR)—the prothrombin time ratio that would result if the World Health Organization international reference for thromboplastin were used to test a blood sample.

Intrahepatic cholestasis—the disorders of hepatocytes and microscopic bile ducts.

Intron—the gene sequence between exons that is excised before mRNA is translated into protein. Introns are historically called "junk" DNA. However, it is increasingly being realized that introns contain gene sequences that do have functional importance.

Inulin—an inert carbohydrate that is filtered by the glomerulus but not reabsorbed or secreted by the renal tubule. It is used to measure glomerular filtration rate.

Invasive test—a test that examines fluids or other substance obtained by penetrating the skin or physically entering the body.

In vitro (literally)—this refers to a reaction or process that occurs inside a test tube.

In vivo (literally)—this refers to a reaction or process that occurs inside the body of a plant or animal or inside cells that are inside the body.

Ischemia—an inadequate blood flow to a part of the body secondary to constriction or blockage of the supplying artery.

Jaundice—yellow discoloration of skin and sclerae due to hyperbilirubinemia. Also known as *icterus*.

Kallmann syndrome—a rare genetic condition in which a male has one or more extra X chromosomes in each cell. This presents with absent or delayed puberty and an impaired sense of smell. In addition, some patients may also have renal agenesis, cleft lip, and cleft palate.

Kernicterus—hyperbilirubinemia-induced brain damage in infants.

Ketonuria—ketones in the urine, which can occur with severe insulin deficiency, starvation, high-fat diets, fever, and anesthesia. Ketones are metabolic products of fat metabolism. The two principal ketones are β-hydroxybutyric acid and acetoacetic acid. Ketonuria is abnormal.

Klinefelter syndrome—a genetic condition in males that presents with small testicles, low serum testosterone levels, decreased muscle mass, sparse body and facial hair, and enlarged breasts.

Km (Michaelis constant)—the concentration of substrate at which an enzymatic reaction rate is half its maximal value.

KRAS—when present, a gene that is strongly associated with primary resistance to the anti-EGFR monoclonal antibodies, panitumumab, and cetuximab.

Lacrimal fluid tears—ultrafiltrate of plasma that is secreted by lacrimal glands in the eye.

Lanugo hair—the down-like, fine, soft hair usually on the ears, forehead, or flank of adult humans.

Laparoscopy—a medical procedure that allows visualization of the abdominal and pelvic organs.

Leukocyte esterase—an enzyme that is released from white blood cells and can be detected in urine by dipstick testing. When present, it indicates the presence of white blood cells in the urine and suggests either infection or inflammation of the urinary tract.

Lower urinary tract symptoms—a term that refers to a collection of urinary obstructive and irritative voiding symptoms, which impacts negatively on a patient's quality of life. Also known as *LUTS*.

Luteal phase—the part of the menstrual cycle in which the secretion of progesterone, rather than estradiol, predominates.

MDRD—modification of diet in renal disease. The original MDRD study was undertaken to assess if reduction in protein intake had beneficial effects on progression of kidney disease. The study used iothalamate clearances to assess glomerular filtration rates. The data was used to develop alternative equations (the MDRD equations) to better identify and treat patients with chronic kidney disease.

MDRD equation—refers to the modification of diet in renal disease formula for estimating glomerular filtration rate in patients with chronic kidney failure. The patient's serum creatinine, age, African-American status, and gender are included in the formula. The MDRD equation tends to underestimate the level of renal function in those with normal or higher levels of renal function.

Mean corpuscular hemoglobin concentration (MCHC)—the average amount of hemoglobin in a red blood cell. A decreased MCHC value implies hypochromic red cells and suggests iron deficiency anemia.

Mean corpuscular volume (MCV)—the average volume of a red blood cell. If the MCV is high, the cells are known as *macrocytic*. This is associated with vitamin B_{12} or folate deficiency. If the MCV is low, the cells are known as *microcytic*. This is associated with iron deficiency.

Meconium—the first discharge of intestinal waste products by a newborn human.

MEK inhibitor—a drug that inhibits mitogen-activated protein kinase. Overactivity of this enzyme is present in some tumors. For example, trametinib is a MEK inhibitor, which may be useful for treatment of BRAF-mutated melanoma.

Menkes syndrome—an X-linked disorder associated with defective copper absorption, which results in stunted growth, mental retardation, defective keratinization and pigmentation of hair, hypothermia, and degenerative changes in the aortic elastin and neurons. Children with this genetic disorder are often deceased by age 3. Also known as *kinky-hair* or *steely-hair syndrome*.

Menses—the bloody discharge that occurs during menstrual cycles.

Metabolic syndrome—a syndrome characterized by abdominal obesity, insulin resistance, hypertension, low levels of high-density lipoprotein cholesterol, and elevations in triglycerides.

Metabolomics—study of metabolic products of cellular processes.

Michaelis–Menten kinetics—a behavior that describes a drug metabolism process that is saturable.

Minimum bactericidal concentration (MBC)—the lowest concentration of a specific antimicrobial agent that kills 99.9% of the inoculum of the organism under a standardized set of in vitro conditions.

Minimum inhibitory concentration (MIC)—the lowest concentration of a specific antimicrobial agent that prevents visible growth of the organism after 24 hours under a standardized set of in vitro conditions.

Mittelschmerz—a lower abdominal and pelvic pain that occurs midway through the menstrual cycle.

Mutation—a variation in genomic DNA that occurs in less than 1% of the population. Mutations may be rare or unique to an individual. The types of variation include single-base pair changes, insertions/deletions, repeats, and chromosomal arrangements.

Myocardium—the muscular tissue of the heart.

Myoglobin—low molecular weight heme protein found in cardiac and skeletal muscle.

Myopathy—elevations in creatine phosphokinase accompanied by muscle pain or tenderness.

Nanotechnology—the emerging clinical science involving the interactions of cellular and molecular components, specifically clusters of atoms, molecules, and molecular fragments.

Natriuretic peptide—a peptide that increases renal sodium excretion. There are three types: A, B, and C. Elevated brain natriuretic peptide is associated with congestive heart failure.

Neonate—a full term newborn of 0–28 days postnatal age or a premature neonate whose postmenstrual age is 41–46 weeks.

Nephrogenesis—development of the kidney.

Nephrotic syndrome—a condition caused by damage to the glomeruli of the kidneys and characterized by large amounts of protein in the urine (proteinuria), low amounts of protein in the blood (hypoproteinemia), edema, and high amounts of cholesterol in the blood (hypercholesterolemia).

Neural tube defect—an abnormal intrauterine development of the spinal cord or brain results in a portion of the spinal cord exposed through the vertebrae (spina bifida), or part of the brain exposed through the skull in the newborn. Some neural tube defects are fatal.

Noninvasive test—a test that examines fluids or other substances obtained without penetrating the skin or physically entering the body.

Nonlinear kinetics—see *Michaelis–Menten*.

Non-ST-segment elevation (myocardial ischemia)—a classification for myocardial ischemia or acute myocardial infarction based on electrocardiogram findings. In this case, there is no ST-segment elevation; instead, there is T-wave inversion or ST depression, which indicates myocardial ischemia but not infarction. This is considered a less severe type of myocardial infarction than an ST-segment elevation myocardial infarction.

Normal flora—the natural colonization of several anatomic sites by bacteria that do not typically cause infection but may become pathogenic under certain circumstances. Normal flora colonization commonly provides defense against invasion by other bacterial or fungal organisms by occupying space, competing for nutrients, and stimulating antibody production.

Oligoarthritis—arthritis that affects one to four joints during the first six months of disease.

Oligomenorrhea—an infrequent or very light menstruation in women with previously normal periods.

Omphalocele—a defect in the wall of the abdomen at the umbilical ring, which occurs during fetal development (i.e., a congenital malformation or birth defect). It allows the intestines (and sometimes other organs) to protrude into the base of the umbilical cord. Thus, the intestines and other organs are outside of the abdominal cavity enclosed in a clear membranous sac. Having the internal organs outside of the abdominal wall will increase insensible water loss.

Oncogene—a gene that normally directs cell growth. If altered, an oncogene can promote or allow the uncontrolled growth of cancer. Alterations can be inherited or caused by an environmental exposure to carcinogens.

Opisthotonus—a spasm of the axial muscles of the spinal column results in this extrapyramidal movement in which the head, neck, and spine of the patient assume an arch-like or bridge-like position. This is a classic presentation of tetanus.

Orchiectomy—the surgical removal of the testes.

Osmolality—a measure of the number of dissolved particles per unit of water. As it pertains to serum, the osmolality is an estimate of the water-solute ratio in vascular fluid. The normal serum osmolality is 270–300 mOsm/kg water. As it pertains to urine, the osmolality is an estimate of the water–solute ratio in urine. The normal urine osmolality is 500–800 mOsm/L.

Osmometry—a process of measuring the osmotic strength of a fluid or substance, which is dependent on the total concentration of solute particles per kilogram.

Osteomalacia—a bone disorder in which bones are soft and weak due to deficiency of vitamin D, calcium, and phosphorus. In children, this is also known as *rickets*.

P wave—the electrocardiogram recording of the electrical activity of the heart leading to atrial depolarization and contraction.

Paget disease—a bone disorder associated with excessive bone resorption and excessive bone formation, which leads to thickened, softened bone. Also known as *osteitis deformans*.

Panhypopituitarism—a disease cause by absent or deficient anterior pituitary gland function, which results in deficiency in growth hormone, luteinizing hormone, follicle-stimulating hormone, adrenocorticotropin, and thyroid-stimulating hormone. A patient may present with clinical symptoms and signs due to one or more hormone deficiencies. This disorder may be due to a disorder of the hypothalamus or the pituitary gland.

Pathogen—a microorganism that is capable of damaging host tissues and eliciting specific host responses and symptoms consistent with an infectious process.

Patient self-management—for patients on chronic warfarin therapy; patients test their own INR and adjust their own therapy, usually based on an algorithm. This approach allows the patient to have more autonomy and control over their dosage regimen.

Patient self-testing—for patients on chronic warfarin therapy; a patient will test his or her own INR but relies on a clinician for interpretation of results and any modifications to the current regimen.

Peak expiratory flow rate—the maximum airflow rate on exhalation. It is measured using a hand-held peak flow meter. If the peak expiratory flow rate is low, it indicates large airway obstruction or that asthma is severe.

Pelvic inflammatory disease—disease of the female reproductive organs (ovary, fallopian tubes, uterus, cervix, vagina) commonly associated with chlamydia or gonorrhea infection; can lead to infertility or ectopic pregnancy.

Perfusion—the circulation of blood through an organ, or the movement of blood through a vascular bed of tissue.

Perimenopause—the period of waning ovarian function that occurs before menopause.

Pharmacoenhancer—a drug that, when coadministered with another drug, increases serum levels of the latter with the objective of increasing and prolonging its effect.

Pharmacogenetics—the translational science of correlating interindividual genetic variation with variability in drug response. This science has the potential to provide personalized medicine selection and dosing to individual patients.

Phenotype—physical presentation of a genetic trait, e.g., hair or eye color, or CYP 450 enzyme capacity to metabolize a particular drug.

Philadelphia (Ph) chromosome—an abnormality of chromosome 22 in which part of chromosome 9 is translocated to it. Bone marrow cells that contain the Ph chromosome are often found in chronic myelogenous leukemia. See *translocation*.

Photometry—a method of laboratory testing in which the laboratory instrument measures the absorbance or emittance of light.

Pica—abnormal food craving.

Plethysmography—as it refers to pulmonary assessments, plethysmography measures lung volumes (or the amount of gas contained in the lungs) at various stages of inflation.

Pluripotential stem cells—embryonic cells that have the ability to differentiate into a variety of different types of specialized cells.

Poikilocytosis—the variability in the circular, biconcave shape of erythrocytes.

Point-of-care testing—the analysis of specimens, involving portable analyzers, that takes place in a physician's office, in emergency rooms, or at the bedside in a patient's home.

Polycystic ovary syndrome—a common endocrine disorder that causes infertility in women. Clinical manifestations include enlarged ovaries with fluid-filled follicular cysts, irregular menstrual cycles, excess body hair, acne, obesity, and elevated serum testosterone and luteinizing hormone levels.

Polymerase chain reaction (PCR)—a clinical laboratory technique involving the in vivo replication and amplification of DNA fragments.

Polymorphism—a variation in DNA that occurs in at least 1% of the population. Examples of types of polymorphisms include single nucleotide polymorphism (single base pair substitutions), insertion/deletions (In/Del; regions of the genome that are inserted or deleted), tandem repeats (a small number of base pairs that are repeated a variable number of times [e.g., TA repeat]), and copy number variants (large regions of the genome or whole genes that occur with variable repetition throughout the genome).

Polyps—small growths in the mucous membrane lining of the uterus, gastrointestinal tract, or nasal sinuses.

Positron emission tomography (PET)—nuclear imaging technique that measures blood flow and cellular metabolism in an organ.

Postnatal age—chronological age since birth.

Prealbumin—a plasma protein similar to albumin but with a shorter half-life. It is synthesized in the liver and is regarded as the best laboratory test of protein malnutrition.

Preanalytic variable—a substance present in the laboratory specimen that interferes with laboratory analytic methods. Examples of such substances include certain drugs, hemolyzed red blood cells, bilirubin, and high lipid concentrations.

Precision—assay reproducibility.

Predictive value—a value that assesses a test's reliability.

Preeclampsia—a condition that occurs in pregnant women characterized by hypertension, edema, and large amounts of protein in the urine. Preeclampsia may lead to eclampsia (an even more serious condition).

Premature neonate—a neonate born at less than 38 weeks gestational age.

Prerenal azotemia—kidney dysfunction caused by a reduced perfusion to the kidney, which could be due to volume depletion (diuretics), hypotension, heart failure, and emboli to the renal arteries. This is characterized by an abnormally high blood urea nitrogen:serum creatinine ratio.

Primary biliary cirrhosis—a chronic disease involving progressive destruction of small intrahepatic bile ducts leading to cholestasis and progressive fibrosis over a period of decades.

Procalcitonin—a precursor of calcitonin. Plasma procalcitonin levels may increase in the presence of acute or chronic inflammation, trauma, or infection.

Prolactinoma—a pituitary that secretes prolactin.

Prostacyclin—a protein that is produced by cells of blood vessel walls and inhibits platelet aggregation.

Protected specimen brush—refers to an invasive procedure to obtain sputum from the lung. A plastic tube that contains a retractable brush is inserted down the throat to the lungs to avoid contaminating the brush with bacteria in the mouth and throat.

Proteinuria—the loss of protein in the urine, which is usually characteristic of glomerular disease.

Proteomics—study of proteins that are produced by organisms.

Pulmonary compliance—the degree of elasticity or stiffness in the lung expressed as the change in volume divided by the change in pressure.

QRS complex—the electrocardiogram recording of the electrical activity of the heart leading to ventricular depolarization and contraction.

Qualitative test—a test whose results are reported as either positive or negative without further characterization of the degree of positivity or negativity.

Quantitative test—a test whose results are reported as an exact numeric measurement (usually a specific mass per unit measurement) and assessed in the context of a reference range.

Red blood cell distribution width (RDW)—a laboratory test that indicates the variability in the size of red blood cells. A high RDW indicates a large variability in size, which often occurs in nutritional anemias and thalassemias.

Reference range—a statistically-derived numerical range of values obtained by testing a sample of individuals assumed to be healthy; represents the range of values where 95% of individuals within the reference population fall.

Renal tubular acidosis—a condition in which the kidney tubules are not able to adequately remove acids from the blood and excrete them in the urine. This decreased ability of the kidney to excrete acids results in a buildup of acids in the blood (metabolic acidosis) and electrolyte imbalances.

Repolarization—an electrical phenomenon that represents the recovery of the resting state electrical potential across membranes of muscle or nerve cells. The intracellular space becomes more negatively charged than the extracellular space leading to cellular relaxation.

Resistant (R)—interpretive category for in vitro susceptibility testing of bacteria where the minimum inhibitory concentration (MIC) of the bacteria is high, and the organism is not likely to be inhibited or eradicated by standard doses of the antimicrobial because the MIC is higher than what can be achieved using maximum doses of the antibiotic.

Reticulocyte—premature red blood cell.

Retrocollis—a dystonia in which sustained muscle contraction causes the head to tilt backward.

Rhabdomyolysis—a condition characterized by breakdown of skeletal muscle tissue with release of myoglobin, enzymes, and electrolytes from cells.

Rheumatoid factors—immunoglobulins directed against the Fc region of immunoglobulin G that are found in the serum of patients with rheumatoid arthritis and other rheumatic diseases.

Rickets—a bone disease caused by chronic vitamin D deficiency and calcium deficiency. Bones become soft and weak.

Rotor syndrome—an autosomal recessive genetic disorder in which patients have increased levels of plasma conjugated and unconjugated bilirubin, icterus, and jaundice.

RT-PCR—reverse-transcriptase polymerase chain reaction. It is a very sensitive molecular genetic test for finding specific DNA sequences, such as those occurring in some cancers. The RNA strand is first reverse transcribed into complementary DNA, followed by amplification of the resulting DNA using a polymerase chain reaction.

Rumack-Mathew nomogram—a semilogarithmic plot of serum acetaminophen concentration versus time, which is used to determine whether there is a need to administer acetylcysteine to reduce the risk of toxicity.

Sarcoidosis—a systemic granulomatous disease affecting many organs. Small nodules of tissue composed of lymphocytes and macrophages appear in skin, lungs, joints, and lymph nodes. Patients may be asymptomatic or develop complications such as pericarditis or meningitis. The etiology is unknown. The disease may or may not be chronic. Also known as *sarcoid*.

Sarcopenia—gradual loss of muscle mass, strength, and function with aging.

Semiquantitative test—a test whose results are reported as either negative or with varying degrees of positivity but without exact quantification.

Sensitivity—when referring to a test, it is the ability of the test to show positive results in patients who actually have the disease (true positive rate). For a test with high sensitivity for a diagnosis of a disease, a patient with a negative test result probably does not have the disease.

Septum—a wall or separation between two compartments.

Serology—a branch of science that deals with measuring specific immunologic responses in the serum or blood to pathogens or foreign substances.

Smith antibodies—a type of antinuclear antibody that is directed at a series of nuclear proteins complexed with small nuclear RNAs. Smith antibodies have high specificity for systemic lupus erythematosus and are more commonly found in African American and Asian patients.

Specificity—the ability of the test to show negative results in patients who do not have the disease (true negative rate). For a test with high specificity for diagnosis of a disease, a patient with a positive test result has a high probability of having the disease.

Specimen—the sample used for laboratory analysis (e.g., whole blood, arterial blood, urine, stool).

Spectrophotometry—a method of laboratory testing in which analyzers measure the intensity of light as a function of wavelength as the light beam is transmitted or reflected through a solution or a see-through solid.

Spirometry—a type of pulmonary function test that measures the maximum amount of air that is exhaled by a patient after complete inhalation.

Sporangiophore—a threadlike structure of a fungus that has sporangia (asexual spores) at the tip.

ST-segment elevation (myocardial infarction)—a classification for myocardial ischemia or acute myocardial infarction based on electrocardiogram findings. In this case, there is ST-segment elevation, which signifies myocardial injury, likely reflecting a degree of cellular damage beyond that of mere ischemia. This is considered a more severe type of myocardial infarction than a non-ST-segment elevation myocardial infarction.

Static spirometry—a pulmonary breathing test that is volume-based and slow.

Susceptible (S)—interpretive category for in vitro susceptibility testing of bacteria where the organism is readily inhibited based on the minimum inhibitory concentration (MIC) of the antibiotic; the organism/infection will most likely be eradicated using standard dosing of the antimicrobial agent for that infection type since concentrations of the antibiotic in the serum and at the site of infection readily exceed the MIC.

Syndrome of inappropriate antidiuretic hormone secretion (SIADH)—patients with SIADH have excessively high levels of antidiuretic hormone, which results in increased water reabsorption and dilutional hyponatremia.

Synovial fluid—the joint fluid that lubricates and nourishes the articular cartilage.

T wave—the electrocardiogram recording of the electrical activity of the heart leading to ventricular repolarization and relaxation.

Tachyzoites—a rapidly reproducing stage of *Toxoplasma gondii*, associated with acute infections.

Thalessemia—a genetic hemoglobinopathy in which the patient has difficulty producing intact hemoglobin inside red blood cells. As a result, the red blood cell is degraded more rapidly and has a shorter lifespan than usual. Thalassemia results in anemia.

Theca cells—cells that produce androgens and progesterone in the ovarian follicle.

Thiopurine methyltransferase (TPMT)—an enzyme responsible for in vivo conversion of azathioprine and 6-mercaptopurine to inactive metabolites. Genetic variants to the TPMT gene can result in deficient or absent TPMT activity, which can lead to increased hematologic adverse effects of azathioprine or 6-mercaptopurine.

Thrombocytopenia—a reduction in the platelet count.

Thrombocytosis—an elevation in the platelet count.

Thromboxane—a protein produced by platelets that is essential for platelet aggregation.

Thyrotoxicosis—a condition due to excessive thyroid hormone also known as hyperthyroidism.

Tissue plasminogen activator—it converts plasminogen to plasmin, which can dissolve blood clots.

Total body water—it includes both extracellular water (in the vascular compartment and interstitial water) and intracellular water volumes.

Toxicokinetics—the pharmacokinetics of drugs and chemicals in the face of overdose.

Transesophageal echocardiography—a sonogram image that is produced from a transducer at the end of flexible endoscope, which is passed through the esophagus and positioned close to the heart.

Transferrin—an iron transporting protein in the blood stream. The percentage of iron-binding sites of transferrin, which are occupied by iron, is known as *transferrin saturation*. This is used as an indirect measure of circulating iron levels.

Translocation—movement of part of one chromosome that has broken off to another chromosome.

Transposon—refers to a portion of a gene that can change positions within a genome, which can result in bacterial resistance to antibiotics or other unexpected phenotypic presentations. Also known as a *disposable element*.

Transthoracic echocardiography—a sonogram image that is produced from a transducer, which is placed on the anterior chest wall.

Transthyretin—a plasma protein similar to albumin but with a shorter half-life. Also known as *prealbumin.*

Transudate—a solute or fluid that passes through a capillary membrane as a result of osmotic pressure or a hydrostatic pressure gradient. Transudates typically have low protein or cellular content.

Triglycerides—an esterified form of glycerol and fatty acids that constitute the main form of lipid storage in humans that is used as fuel for gluconeogenesis or for direct combustion as an energy source.

Troponin—a protein that regulates calcium-mediated interaction of actin and myosin, essential for contraction of cardiac muscle.

Trousseau's sign—a sign of latent tetany due to hypocalcemia. Carpal spasm is induced by inflating a blood pressure cuff on the ipsilateral arm for three minutes.

Tumor marker—substances produced by tumor cells or by other cells of the body in response to cancer. These substances can be found in the blood, urine, tumor tissue, or other tissues. Some tumor marker levels can also be altered in patients with noncancerous conditions, which limit their usefulness for cancer screening.

Turbidimetry—a technique for measuring the percent light absorbed as light passes through a solution. As turbidity increases, the intensity of the light beam as it passes through the particles in the solution decreases.

Tyrosine kinase—an intracellular enzyme that transfers a phosphate group from adenosine triphosphate to a tyrosine residue in a protein. Phosphorylation of proteins by kinases is an important mechanism in signal transduction (and cell growth) and often becomes dysregulated in cancer.

U-waves—refers to a portion of an electrocardiograph tracing. U-waves normally follow T-waves and are in the same direction as T-waves. The U-wave represents repolarization of Purkinje fibers. U-waves are more often visible when the heart rate is <65 beats/minute or in the face of hypokalemia.

Ultrafiltrate—a solution that has passed through a semipermeable membrane with very small pores.

Urethral stricture—scarring of the urethra (due to infection, inflammation, or instrumentation) that results in narrowing of the urethral lumen. A patient will then have difficulty passing urine from the bladder through the narrowed urethral lumen.

Uridine glucuronosyltransferase—an enzyme responsible for conjugating bilirubin in the liver so that it can be excreted renally.

Urobilinogen—bilirubin that is metabolized by intestinal bacteria to urobilinogen. Although most urobilinogen undergoes hepatic recirculation, a small amount is normally excreted in urine. When urinary urobilinogen levels are high, it suggests excessive red blood cell turnover, as would occur with hemolytic anemia, or hepatic injury.

Urokinase—produced by the kidney and can convert plasminogen to plasmin, which can dissolve clots. It is used as a pharmaceutical agent. Also known as *urine plasminogen activator.*

Vasopressin—it enhances water reabsorption at the collecting duct portion of the renal tubule. Also known as *antidiuretic hormone*.

Ventilation—the movement of air in and out of the lungs.

VKORC1—a gene that encodes for vitamin K epoxide reductase complex subunit 1, the enzyme responsible for activating vitamin K. Various VKORC1 genotypes affect the daily dose of warfarin and potential of bleeding with warfarin in patients. Uncommonly, a VKORC1 genotype is associated with warfarin resistance.

V_{max}—the maximum rate of a drug's metabolism by a particular enzyme system in the liver.

Von Willebrand factor—a circulating protein that binds to other circulating proteins and is essential for platelet adhesion.

Wilson disease—an autosomal recessive disease of improper copper storage. It is associated with elevated urinary copper loss; low plasma ceruloplasmin; low copper concentrations; and copper deposition in the liver, brain, and cornea.

Zero-order bioavailability or elimination rate—The absorption or elimination rate of a drug from the body proceeds at a constant rate and is saturable. The rate of absorption or elimination is independent of plasma drug concentration.

APPENDICES

APPENDIX A. Therapeutic Ranges of Drugs in Traditional and SI Units[a]

DRUG	TRADITIONAL RANGE	CONVERSION FACTOR[b]	SI RANGE
Acetaminophen	75 mg/dL toxic	66.16	7330 µmol/L
N-acetylprocainamide	4–10 mg/L	3.606	14–36 µmol/L
Amikacin	20–30 mcg/mL	1.708	34–51 µmol/L
Amiodarone	0.5–2.5 mcg/mL	1.55	0.8–3.9 µmol/L
Amitriptyline	120–250 ng/mL	3.605	433–901 nmol/L
Carbamazepine	4–12 mg/L	4.23	17–51 µmol/L
Chlordiazepoxide	0.5–5 mg/L	3.336	2–17 µmol/L
Chlorpromazine	50–300 ng/mL	3.136	150–950 nmol/L
Chlorpropamide	75–250 mcg/mL	3.613	270–900 µmol/L
Clonazepam	10–50 ng/mL	0.317	3.2–15.9 nmol/L
Clozapine	200–350 ng/mL	0.003	0.6–1 µmol/L
Cyclosporine	100–400 ng/mL[c]	0.832	83–333 nmol/L
Desipramine	100–160 ng/mL	3.754	375–600 nmol/L
Diazepam	100–1000 ng/mL	0.0035	0.35–3.5 µmol/L
Digoxin	0.9–2.2 ng/mL	1.281	1.2–2.8 nmol/L
Disopyramide	2–6 mg/L	2.946	6–18 µmol/L
Doxepin	50–200 ng/mL	3.579	180–720 nmol/L
Ethosuximide	40–100 mg/L	7.084	280–710 µmol/L
Fluoxetine	200–1100 ng/mL	0.00323	0.65–3.56 µmol/L
Gentamicin	6–10 mcg/mL	2.09	12.5–21 µmol/L
Glutethimide	>20 mg/L toxic	4.603	>92 µmol/L toxic
Gold	<10 mcg/L	50.77	<508 nmol/L
Haloperidol	5–15 ng/mL	2.66	13–40 nmol/L
Imipramine	200–250 ng/mL	3.566	710–900 nmol/L
Isoniazid	>3 mg/L toxic	7.291	>22 µmol/L toxic
Lidocaine	1–5 mg/L	4.267	5–22 µmol/L
Lithium	0.5–1.5 mEq/L	1	0.5–1.5 mmol/L
Meperidine	400–700 ng/mL	4.043	1617–2830 nmol/L
Methotrexate	>2.3 mg/L toxic	2.2	>5 µmol/L toxic
Nortriptyline	50–150 ng/mL	3.797	190–570 nmol/L
Pentobarbital	1–5 mcg/mL	4.439	4–22 µmol/L
Phenobarbital	15–40 mg/L	4.306	65–172 µmol/L
Phenytoin	10–20 mg/L	3.964	40–80 µmol/L
Primidone	4–12 mg/L	4.582	18–55 µmol/L
Procainamide	4–8 mg/L	4.249	17–34 µmol/L
Propoxyphene	>500 ng/mL toxic	2.946	>1500 ng/mL
Propranolol	50–200 ng/mL	3.856	190–770 nmol/L
Protriptyline	70–250 mcg/dL	3.787	265–947 nmol/L

(continued)

APPENDIX A. Therapeutic Ranges of Drugs in Traditional and SI Units[a], cont'd

DRUG	TRADITIONAL RANGE	CONVERSION FACTOR[b]	SI RANGE
Quinidine	2–6 mg/L	3.082	5–18 μmol/L
Salicylate (acid)	150–300 mcg/mL	7.24	1086–2172 μmol/L
Theophylline	10–20 mg/L	5.55	55–110 μmol/L
Tobramycin	5–10 mcg/mL	2.139	10.7–21 μmol/L
Tocainide	4–10 mcg/mL	5.201	21–52 μmol/L
Valproic acid	50–100 mg/L	6.934	350–700 μmol/L
Vancomycin	20–40 mcg/mL	0.69	14–28 μmol/L

[a] Also see Table 5-3 in Chapter 5.

[b] Traditional units are multiplied by conversion factor to get SI units.

[c] Whole blood assay.

APPENDIX B. Nondrug Reference Ranges for Common Laboratory Tests in Traditional and SI Units[a,b]

LABORATORY TEST	REFERENCE RANGE TRADITIONAL UNITS	CONVERSION FACTOR	REFERENCE RANGE SI UNITS	COMMENT
Alanine aminotransferase (ALT)	0–30 IU/L	0.01667	0–0.5 μkat/L	SGPT
Albumin	3.5–5 g/dL	10	35–50 g/L	
Alkaline phosphatase	30–120 units/L	0.0167	0.5–2 μkat/L	
Ammonia (as nitrogen)	15–45 mcg/dL	0.714	11–32 μmol/L	
Aspartate aminotransferase (AST)	8–42 IU/L	0.01667	0.133–0.7 μkat/L	SGOT
Bilirubin (direct)	0.1–0.3 mg/dL	17.1	1.7–5 μmol/L	
Bilirubin (total)	0.3–1 mg/dL	17.1	5–17 μmol/L	
Calcium	8.5–10.8 mg/dL	0.25	2.1–2.7 mmol/L	
Carbon dioxide (CO_2)	24–30 mEq/L	1	24–30 mmol/L	Serum bicarbonate
Chloride	96–106 mEq/L	1	96–106 mmol/L	
Cholesterol (HDL)	>40 mg/dL	0.026	>1.05 mmol/L	Desirable
Cholesterol (LDL)	<130 mg/dL	0.026	<3.36 mmol/L	Desirable
Creatine kinase (CK)	25–90 IU/L (males)	0.01667	0.42–1.5 μkat/L	Males
	10–70 IU/L (females)		0.17–1.17 μkat/L	Females
Creatinine, serum (SCr)	0.7–1.5 mg/dL	88.4	62–133 μmol/L	Adults
Creatinine clearance (CrCl)	90–140 mL/min/1.73 m^2	0.017	1.53–2.38 mL/sec/1.73 m^2	
Folic acid	3–16 ng/mL	2.266	7–36 nmol/L	
γ-glutamyl transpeptidase (GGT/GGTP)	0–30 units/L (but varies)	0.01667	0–0.5 μkat/L (but varies)	GGT/GGTP
Glucose (fasting)	70–110 mg/dL	0.056	3.9–6.1 mmol/L	Fasting
Hemoglobin (Hgb)	14–18 g/dL (males)	0.622	8.7–11.2 mmol/L	Males
	12–16 g/dL (females)	0.622	7.4–9.9 mmol/L	Females
		10	140–180 g/L	Males
		10	120–140 g/L	Females
Iron	50–150 mcg/dL	0.179	9–26.9 μmol/L	
Lactate (arterial), serum	0.5–2 mEq/L	1	0.5–2 mmol/L	
Lactate (venous), serum	0.5–1.5 mEq/L	1	0.5–1.5 mmol/L	Lactic acid
Lactate dehydrogenase (LDH)	100–210 IU/L	0.01667	1667–350 nmol/L, 1.7–3.2 μkat/L	LDH
Magnesium	1.5–2.2 mEq/L	0.5	0.75–1.1 mmol/L	
5' nucleotidase	1–11 units/L (but varies)	0.01667	0.02–0.18 μkat/L (but varies)	

APPENDIX B. Nondrug Reference Ranges for Common Laboratory Tests in Traditional and SI Units[a,b]

LABORATORY TEST	REFERENCE RANGE TRADITIONAL UNITS	CONVERSION FACTOR	REFERENCE RANGE SI UNITS	COMMENT
Phosphate	2.6–4.5 mg/dL	0.3229	0.85–1.48 mmol/L	
Potassium	3.5–5 mEq/L	1	3.5–5 mmol/L	
Sodium	136–145 mEq/L	1	136–145 mmol/L	
Thyroxine (T_4), total serum	5–12 mcg/dL	12.86	64–154 nmol/L	Total T_4
Total iron-binding capacity (TIBC)	250–410 mcg/dL	0.179	45–73 μmol/L	TIBC
Triglycerides	<150 mg/dL	0.0113	<1.26 mmol/L	Adults >20 yr
Triiodothyronine (T_3), total serum	78–195 ng/dL	0.0154	1.2–3 nmol/L	Total T_3
Urea nitrogen, blood (BUN)	8–20 mg/dL	0.357	2.9–7.1 mmol/L	BUN
Uric acid (serum)	3.4–7 mg/dL	59.48	202–416 μmol/L	

SGOT = serum glutamic oxaloacetic transaminase; SGPT = serum glutamic pyruvic transaminase.

[a] Some laboratories are maintaining traditional units for enzyme tests.

[b] For more extensive listing, refer to www.amamanualofstyle.com/page/si-conversion-calculator (accessed 2016 Mar 1).

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